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PATTERNS OF CELL DEATH IN STALKS OF NORMAL AND INJURED SORGHUM

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ABSTRACT. — The effects of root injury, removal of the distal two-thirds of all leaves, and removal of inflorescences on cell death were studied in four sorghum varieties (De Kalb C44B, C45, E56A, and F63). The removal of inflorescences decreased the rate of cell death in the stalk tissue while both removal of the distal two-thirds of all leaves and root injury increased the rate. This model system which provides methods of modifying cell death rates will be useful in the study of the role of living cells in resistance to spread of fungal pathogens in sorghum stalks, and in the study of the cell death process in normal and diseased plants.

Katsanos and Pappelis (1965) studied the patterns of cell death in the lower stalk internodes of six varieties of grain sorghum and reported an increase in cell death after inflorescences emerged. The study was continued for two months. Internodal and nodal stalk tissue which macroscopically appeared white and fluffy when the stalks were cut longitudinally through the central axis, microscopically was found to be composed of dead, air-filled cells with no evidence of the protoplast. However, internodal and nodal stalk tissue that macroscopically appeared well hydrated and light cream in color, microscopically was found to be composed of living cells with protoplasts that could be plasmolyzed. Pappelis and Katsanos (1966) reported that root and leaf injury increased the rate of cell death in internodal and nodal tissue, whereas removal of the inflorescence decreased the rate of cell death. The study was limited to two varieties (De Kalb C44B and E56A). In the same year, Katsanos and Pappelis made two reports (1966a, 1966b) that *Colletotrichum graminicola* (Ces.) G. W. Wils in inoculated plants of three varieties (De Kalb C44B, E56A, and F63) and in stalks of root-injured plants of one variety (De Kalb F63) spread through areas of dead cells and was inhibited by living cells or factors associated with them. Since it is important to develop model systems to test the hypothesis that living cells are involved in disease resistance in stalk rot, the present investigation was conducted to verify and extend the information on the effect of injury on cell death.

MATERIALS AND METHODS

(De Kalb C44B, C45, E56A, and F63) four varieties of grain sorghum, were planted in a three replicate experiment on May 16, 1965, on Southern Illinois

University Agronomy Farm, Carbondale. In each replicate, each variety was planted in plots of 24 rows spaced 100 cm apart, each row containing 40 plants spaced 15 cm apart.

The inflorescences emerged in more than 50% of the plants in each replicate on the following dates: E56A, July 18; C44B, July 20; C45, July 22; and F63, July 24. On the above dates, the plots were randomly divided into sub pots and the following treatments applied: normal; inflorescence removed as they emerged; distal two-thirds of all leaves removed; and roots cut by inserting a 15 cm wide spade, starting about 10 cm away from the stalk and pushing down at a 45 degree angle to intersect below the stalk. Roots were cut a second time three weeks later. Since weed control was by chemical means (Atrazine) and hand hoe, the spade patterns were easily observed in the soil, and the spade was inserted at the same place on the second date of root cutting.

Each variety was sampled seven times at biweekly intervals starting on the date the treatments were applied. On each day, the four elongated internodes above the uppermost adventitious roots of 10 plants in each sub-plot were rated by splitting the stalks longitudinally and rating the amount of dead (white, fluffy) internodal and nodal tissue using the following system: 0.0, no white and fluffy in the internode; 0.1, less than 1% white; 0.5, 2 to 12%; 1, 13 to 25%; 2, 26 to 50%; 3, 51 to 75%; 4, 76 to 100%; and 6, plant dead. When dead tissue in a node linked dead tissue in adjacent internodes, the letter T was added to the rating of the lower internode. For each sampling day, the pith condition ratings for each internode of all 10 plants of every treatment in each replicate of a variety were averaged. The mean for the three replicate averages for each internode was determined and will be referred to as pith condition field average.

Results

The field averages for pith condition ratings are presented in Table 1. In all treatments of every variety, the pith condition ratings increased with time. During the greater part of the study, pith condition ratings of all treatments were progressively lower from first to fourth internodes for E56A, but at the end of the study the ratings became progressively higher. For variety C44B, ratings were progressively higher from first to fourth internodes for all treatments during the entire study. For varieties C45 and F63, pith ratings were progressively lower from first to fourth internodes for all treatments during the entire study. In general, C44B showed the greatest increase of pith ratings for all treatments, followed in order by C45, F63, and E56A.

Leaves of root-cut plants of all varieties showed wilt symptoms within one hour after cutting. Within two days, the lower two or three leaves died, tips of some of the upper leaves were curled, and tips of some others had become yellow or brown. A few plants of each variety died. After this time, no other premature death of leaves or entire plants was observed, but all plants of this treatment generally appeared less healthy than the plants of the other treatments.

In all varieties, the root-cut treatment increased the pith condition ratings, that is, it increased the rate of cell death more than any other treatment. This increase was obvious early in the study, but it became most pronounced during the latter part. Root cutting also resulted in the greatest amount of death of cells in the nodes as indicated by the presence of the letter T in Table 1. It should be noted that normal nodal cell death in these varieties studied appears later in the season, occurring after many internodal cells have died.

In the leaf-cut treatment, plants of varieties F63 and C44B appeared less green and healthy than those of

TABLE 1.—Field averages[a] for pith condition (cell death) ratings[b] in normal stalk tissue of sorghum and those injured as follows: inflorescence removed (Head-cut), distal two-thirds of all leaves removed (Leaf-cut), and roots on both sides of the stalk cut on two dates using a spade 15 cm in width (Root cut).

Treatment and Variety	Internodes	Pith Condition Ratings on Sample Dates[c]						
		1	2	3	4	5	6	7
E56A								
Normal	4	0.0	0.3	0.6	1.7	2.0	2.8	3.0
	3	0.0	0.4	0.9	1.9	2.3	2.8	3.0
	2	0.2	0.5	1.1	2.2	2.5	2.8	2.5
	1	0.5	0.6	1.2	2.4	2.5T	2.5	2.5
Head-cut	4	.....	0.1	0.8	1.9	2.0	2.1	1.8
	3	.....	0.3	1.2	2.0	2.0	2.1	1.9
	2	.....	0.5	1.2	1.8	1.6	1.6	1.6
	1	.....	0.8	1.0	1.4	1.3	1.2	1.4
Leaf-cut	4	.....	0.2	0.7	1.8	2.2	2.6	3.2
	3	.....	0.3	0.9	1.9	2.5	2.7	3.2
	2	.....	0.5	1.0	2.1	2.6	2.7	3.1
	1	.....	0.6	1.2	2.0	2.6	2.7	2.8
Root-cut	4	.....	0.4	1.3	2.5	2.7	3.6T	3.9T
	3	.....	0.6	1.5	2.6	2.9	3.7T	3.9T
	2	.....	0.8	1.8	2.9T	3.1T	3.8T	3.8T
	1	.....	0.9	2.0	3.0T	3.1T	3.8T	3.8T
C44B								
Normal	4	2.0	2.8	3.4	3.7T	3.5	3.7T	4.0T
	3	1.7	2.4	3.2	3.7T	3.6T	3.6T	3.9T
	2	1.2	1.6	2.6	3.7T	3.6T	3.6T	3.8T
	1	0.8	1.1	2.2	3.6T	3.6T	3.6T	3.7T
Head-cut	4	.....	3.0	3.2	3.1	3.2	3.2	3.2
	3	.....	2.6	2.5	2.6	2.9	2.7	2.9
	2	.....	1.6	1.8	2.0	2.2	1.8	2.2
	1	.....	1.0	1.2	1.5	1.6	1.5	1.5
Leaf-cut	4	.....	3.1	3.4	3.7T	3.7T	3.9T	4.1T
	3	.....	2.6	3.1	3.7T	3.7T	3.8T	4.1T
	2	.....	1.8	2.7	3.6T	3.6T	3.8T	4.0T
	1	.....	1.4	2.1	3.5T	3.5T	3.8T	3.9T
Root-cut	4	.....	3.5	3.5T	3.8T	3.7T	4.2T	4.5T
	3	.....	3.1	3.3T	3.8T	3.7T	4.2T	4.5T
	2	.....	2.5	3.0T	3.7T	3.7T	4.1Y	4.5T
	1	.....	1.8	3.8T	3.7T	3.7T	4.0T	4.5T
C45								
Normal	4	0.1	0.2	0.9	1.2	2.0	2.1	2.6
	3	0.1	1.4	1.0	1.6	2.2	2.3	2.8
	2	0.1	0.6	1.4	2.0T	2.4T	2.3	2.8
	1	0.2	0.7	1.4	2.2T	2.5T	2.4T	2.8T
Head-cut	4	.....	0.2	0.9	1.0	1.0	1.4	1.5
	3	.....	0.3	0.9	1.1	1.1	1.4	1.5
	2	.....	0.4	1.0	1.3	1.2	1.4	1.6
	1	.....	0.6	1.0	1.4	1.3	1.4	1.6

TABLE 1.—Concluded.

Treatment and Variety	Inter-nodes	Pith Condition Ratings on Sample Dates[c]						
		1	2	3	4	5	6	7
Leaf-cut	4	.....	0.3	0.8	1.1	1.6	2.3	2.2
	3	.....	0.4	0.9	1.4	1.8	2.3	2.3
	2	.....	0.6	1.2	1.7	2.0	2.3	2.4
	1	.....	0.6	1.2	2.0	2.0	2.3	2.4
Root-cut	4	.....	0.8	1.5	1.9	2.4	2.6	4.0T
	3	.....	1.0	1.7	2.2	2.5	2.7	4.1T
	2	.....	1.3	2.0	2.5T	2.8T	2.8T	4.1T
	1	.....	1.3	2.0	2.5T	2.8T	2.8T	4.1T
F63								
Normal	4	0.1	0.5	0.5	0.9	1.3	1.7	2.6
	3	0.1	0.6	0.6	1.2	1.4	1.8	2.7
	2	0.2	0.7	0.8	1.4	1.7	1.8	2.8
	1	0.3	0.8	0.8	1.5	1.9	1.8	2.8
Head-cut	4	.....	0.5	0.7	0.9	0.9	1.2	1.4
	3	.....	0.7	0.9	0.9	1.0	1.2	1.5
	2	.....	0.7	0.9	1.0	1.0	1.1	1.5
	1	.....	0.8	0.9	1.1	1.0	1.1	1.5
Leaf-cut	4	.....	0.4	0.9	1.3	1.7	2.2	2.6
	3	.....	0.6	1.0	1.7	1.9	2.5	2.6
	2	.....	0.7	1.3	2.0	1.9	2.6T	2.9T
	1	.....	0.7	1.4	2.2	2.0	2.7T	2.9T
Root-cut	4	.....	0.9	1.2	1.7	2.5	3.0T	4.1T
	3	.....	1.0	1.3	2.2	2.6	3.0T	4.1T
	2	.....	1.3	1.5	2.4	2.7	3.2T	4.1T
	1	.....	1.4	1.5	2.5	2.8T	3.2T	4.1T

a. Field averages are the means of three replicate averages based on 10-plant samples in each treatment sub-plot.
b. Pith condition ratings: 0.0, no white tissue composed of dead cells; 0.1, less than 1% white; 0.5, 2 to 12%; 1, 13 to 25%; 2, 26 to 50%; 3, 51 to 75%; 4, 75 to 100%; and 6, plant dead. When dead tissue in a node linked dead tissue in adjacent internodes, the letter T was added to the rating of the lower internode.
c. Sample dates: E56A, July 18, August 2, 16, 30, September 13, 18, and October 13; C44B, July 20, August 4, 16, 30, September 13, 28, and October 13; C45, July 22, August 5, 19, September 2, 16, 30, and October 15; and F63, July 24, August 6, 19, September 2, 16, 29, and October 15.

the normal and head removal treatments. For these two varieties, leaf cutting resulted in pith condition ratings exceeded only by those of the root-cut treatment. For C45 and E56A, pith ratings in leaf-cut plants were higher than those of normal plants during most of the sampling days, but the differences were not as marked as that observed between plants of the root cutting and normal treatments. For variety E56A, leaf cutting resulted in a slight increase in pith condition ratings during the last three sampling days, whereas during the first three sampling days, rating decreased slightly as compared to those of the normal plants rated on the same days. In variety C45, pith condition ratings in leaf-cut plants were lower than those of the normal plants during the entire study.

Plants with inflorescences removed, appeared greener and healthier dur-

ing the entire period than the plants of the other treatments, including those of the normal one. Inflorescence removal resulted in many auxilliary buds forming vigorously growing new shoots and developing new inflorescences (which were removed as they appeared). Except for a slight increase in some internodes during the first, second, or both sampling days for varieties E56A, C44B, and F63, pith condition ratings were lower than the ratings in the plants of the other treatments. Inflorescence removal prevented nodal cell death during the period of study.

Discussion

The treatment of root and leaf cutting had, in general, the same effect since both increased cell death in the stalks. The reasons for this are probably not the same. Root cutting results in decrease of water uptake, whereas leaf removal would reduce transpiration. Synthesis of carbohydrates and other substances would greatly be reduced by both injuries. The removal of the inflorescence would be expected to increase the level of carbohydrates in the stalk, whereas continued production of new shoots would require carbohydrates. The hormonal balance would be expected to be greatly changed by these injuries. Whatever may be the reasons for modification of the cell death rate resulting from plant injury, the change in rate will provide new model systems to test the effect of the living cells on resistance to spread of fungal stalk rot pathogens in sorghum stalk tissue. Since consistent differences appear between the cell death patterns in each variety, sorghum may prove to be useful in the study of genetics of cell death. Since the pattern of cell death appear to follow predictable patterns within the stalks, the use of this tissue may prove useful in the study of cell death at the cytochemical and ultrastructural level.

Acknowledgments

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Manuscript received June 19, 1968.

OXYGEN ABSORPTION BY COAL. SOME OBSERVATIONS ON OIL TREATMENT

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ABSTRACT. — A sample of Colchester (No. 2) Coal of Illinois, compared with coals previously tested, was found to absorb oxygen at about the same rate as a Harrisburg (No. 5) Coal sample, but appreciably faster than a Herrin (No. 6) Coal sample. Oil-treated No. 2 Coal, after about one week of exposure to oxygen at room temperature, absorbed oxygen more rapidly than did the untreated coal. Oxygen absorption by the oil alone showed an induction period of about one week. Oil-treated coal absorbed oxygen more rapidly than would be predicted by adding together the rates for coal and oil measured separately.

Rates of oxygen absorption and loss of coking properties (i.e., Gieseler fluidity) of coals and the effects of treating the coals with antioxidants were studied a few years ago for samples of Harrisburg (No. 5) Coal and Herrin (No. 6) Coal from Illinois (Yohe, 1965). Neither oxygen absorption nor loss in Gieseler fluidity was found to be appreciably slowed by the application of reasonable amounts of antioxidants to the coals.

After these results had been published (Yohe, 1965), a coal producer mining the Colchester (No. 2) Coal (a high-volatile C bituminous coal that is mined extensively in the northern and western parts of the state's coal-producing area) called attention to the fact that users of this coal were apparently having more frequent trouble with spontaneous heating and ignition of stored coal than were users of certain other coals. He raised two questions: (1) Did this coal absorb oxygen more rapidly than other coals? (2) What effect did oil treatment of the prepared coal have on the rate of oxygen absorption?

A sample of the No. 2 Coal was obtained, as was a sample of the oil used at a preparation plant for "dust-proofing" this coal. Oxygen absorption tests on the coal, the oil, and on oil-treated coal were made under essentially the same conditions that were used previously for the No. 5 and No. 6 Coals. A few determinations of Gieseler fluidities also were made on treated and untreated coal samples.

The No. 2 Coal absorbed oxygen at about the same rate as the No. 5 Coal but more rapidly than the No. 6 Coal. Oil-treated No. 2 Coal, after about one week of exposure, absorbed oxygen more rapidly than did the untreated coal.

MATERIALS USED

A 25-pound gross sample of the No. 2 Coal was obtained at a preparation plant

by taking numerous small portions from a conveyor belt. This coal was of the 1¼ by ¾ inch size range; it was not oil treated, but had been "washed" by the heavy medium (magnetite in water) process. The gross sample was brought to the laboratory, further rinsed with water to remove fines adhering to the pieces, and air dried. A portion of it was ground to -40 mesh for the Gieseler fluidity and oxygen absorption tests, and some was reduced further to -60 mesh for an analytical sample.

Analytical data: Moisture (as received), 11.7%; ash (moisture-free basis), 5.4%; volatile matter and fixed carbon (moisture- and ash-free basis), 49.7% and 50.3%, respectively. Ultimate analysis (moisture- and ash-free basis): H, 5.7; C, 80.3; N, 1.4; O, 9.1; S, 3.5%; calorific value, 14,556 Btu/lb.

A second similar sample obtained 6 months later was analyzed for moisture and ash only: moisture (as received), 9.4%; ash (moisture-free basis), 5.4%.

The oil was described as an 800-viscosity (at 100° F) oil, and was produced by a southern Illinois petroleum company. It was found to have a specific gravity of 1.03.

Procedures and Results

The Gieseler determination is essentially a measurement of the viscosity of the coal as it is heated. Constant torque is applied to a special stirrer imbedded in the coal sample, which is heated at a specified rate. As partial melting occurs, the stirrer turns, and the rate of turning is measured in arbitrary units called dial divisions per minute. An increase in fluidity results in an increased rate of turning; the maximum rate is one of the several items of data recorded and is often considered in evaluating the coking properties of the coal (American Society for Testing and Materials, 1964).

Four 50-gram portions of the -40 mesh coal were weighed into beakers and mixed with 25 ml of chloroform containing, respectively, 0, 0.269, 0.537, and 0.806 grams of oil. These amounts of oil correspond on a weight basis to 0, 5, 10, and 15 quarts of oil per ton of coal, but on the basis of surface area of the coal they would be much less than the amounts applied commercially to the coal. (A cube with edges of unit length has 6 square units of surface; if subdivided into cubes of 1/n unit edge length, the same volume would have 6n square units of surface.)

The chloroform, which was used merely as a solvent to distribute the oil uniformly over the coal surface, was permitted to evaporate, and the treated coal samples were spread out and exposed to the air of the laboratory. Gieseler fluidity determinations were run, as shown in Table 1.

These data (Part A) show the expected loss in fluidity as the coal is subjected to atmospheric oxidation and (Part B) indicate that the oil treatment has given the coal some protection against loss of plastic properties during this comparatively brief exposure. The higher dosages of oil apparently gave slightly greater protection.

Oxygen Absorption Tests

Weighed samples of coal, oil, or oil-treated coal were placed in suitable glass flasks that were attached to gas burets connected to mercury leveling bulbs. The containers were sealed, and the systems filled with oxygen and allowed to stand at room temperature (Yohe, 1965). Five-

Table 1. — Gieseler Maximum Fluidities.

A. Untreated Coal (Control)		B. At 11-12 Days of Exposure	
Days of Exposure	Dial Divisions per Minute	Grams of Oil per 50 g Coal	Dial Divisions per Minute
		0 (Control)	73
1	375	0.269	79
4	146	0.537	96
11-12	73	0.806	132

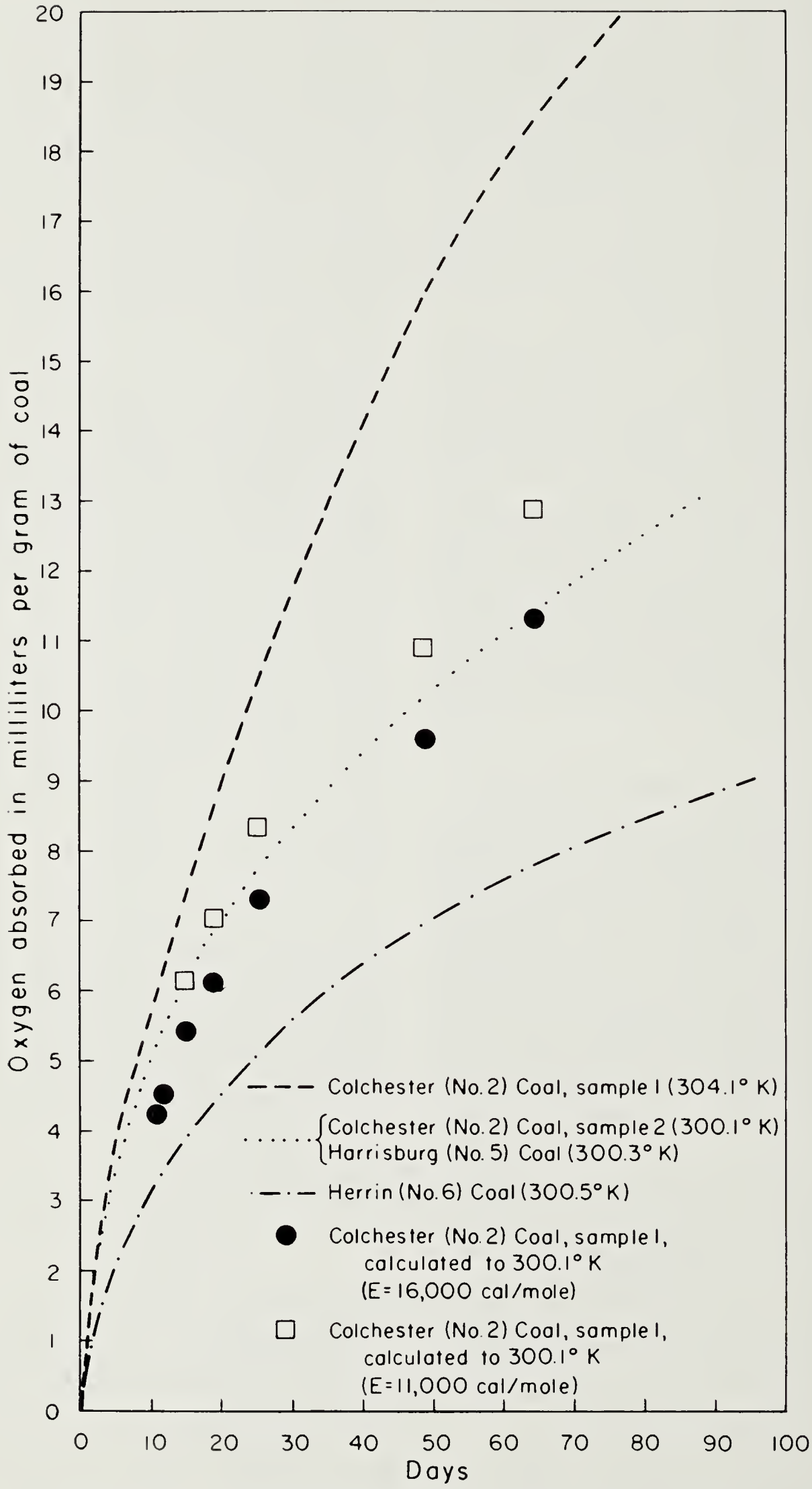

FIGURE 1. — Oxygen absorption by various coal samples.

gram samples of the coals were used; oil samples of 1.29 g were dispersed on -40-mesh silicon carbide or vermiculite, the bulk volume of which was approximately equal to that of 5 g of coal. Readings of temperature, oxygen pressure and volume, and barometric pressure were made periodically, and the results calculated to milliliters of oxygen at standard conditions absorbed per gram of substrate material.

Figure 1 shows the oxygen absorption of two samples of the No. 2 Coal compared with that of the No. 5 and No. 6 Coals previously described (Yohe, 1965).

The greater rate of absorption displayed by sample 1 of the No. 2 Coal is undoubtedly caused by the higher mean ambient temperature (304.1° K compared to 300.1° K for sample 2) at the time the tests were run. Recalculation of these results to comparable temperatures may be made by means of the integrated form of the Arrhenius equation (Schmidt, 1945, p. 642):

$$2.303 \log \frac{R_1}{R_2} = \frac{E}{1.986} \left\{ \frac{1}{T_2} - \frac{1}{T_1} \right\}$$

where R_1 is the reaction rate at absolute temperature T_1, R_2 is the rate at temperature T_2, and E is the energy of activation. Figure 1 also shows the positions of the rate curve for sample 1 of the No. 2 Coal when recalculated to the temperature of the sample 2 experiments, using E = 11,000 and E = 16,000 calories per mole (Schmidt, 1945, pp. 643, 644). The results of these calculations show that the oxygen absorption rates would have been similar if the samples had been tested at the same temperature.

The No. 2 Coal absorbed oxygen at the same rate as the No. 5 Coal, but at an appreciably higher rate than the No. 6 Coal.

Oil-Treated Coal. Two series of tests were made on oil-treated portions of the coal. The first tests (Table 2) were made with oil dosages that approximated treatments used commercially on a weight basis. These dosages, however, were far too light to give adequate sur-

TABLE 2. — Oxygen Absorbed by Sample 1, Colchester (No. 2) Coal (mean ambient temperature, 304.1° K)

mg oil per g coal:	0	5.4	10.7	16.1
Days exposed	ml oxygen per g coal			
0	0	0	0	0
0.6	1.1	1.1	0.8	0.9
1.0	1.5	1.5	1.1	1.2
2.0	2.3	2.6	2.2	2.1
3.6	3.3	3.9	3.5	3.2
4.6	3.9	4.5	4.2	3.7
6.0	4.3	5.3	5.1	4.3
6.6	4.8	5.8	5.7	5.1
8.0	5.4	6.3	6.3	5.5
10.6	6.0	7.7	7.7	6.5
11.6	6.4	7.5	8.3	7.1
15.0	7.7	8.9	9.6	8.6
18.6	8.8	10.3	11.2	9.9
25.0	10.4	11.9	13.0	11.6
48.6	16.0	17.2	18.4	17.2
64.0	18.4	19.3	20.5	19.6
104·6	22.0	22.4	23.7	22.9
110.0	22.4	22.9	24.2	23.5

TABLE 3. — Oxygen Absorbed by Sample 2, Colchester (No. 2) Coal (mean ambient temperature, 300.1° K)

mg oil per g coal:	0	172	258	344
Days exposed	ml oxygen per g coal			
0	0	0	0	0
0.6	1.0	0.7	0.3	0.7
1.6	2.0	1.3	1.0	1.6
2.6	2.4	1.6	1.5	2.2
4.0	3.1	2.4	2.2	2.9
5.6	3.9	2.9	3.1	3.7
11.6	5.2	4.6	5.4	5.8
13.6	5.8	5.4	6.3	6.7
15.6	6.0	5.9	6.8	7.1
17.6	6.7	6.9	7.7	7.9
21.0	7.2	7.6	8.4	8.8
24.6	7.7	8.4	9.3	9.6
31.6	8.5	9.9	11.1	11.3
38.6	9.2	11.2	12.2	12.4
47.6	9.9	12.7	13.7	13.8
52.6	10.5	13.6	14.5	14.6
62.6	11.3	14.8	15.5	15.7
69.6	11.8	15.7	16.3	16.4
82.6	12.7	17.1	16.9	17.3
84.6	12.7	17.2	17.0	17.4
92.0	13.2	17.8	17.7	18.0
103.0	13.7	18.5	18.1	18.4
141.0	15.4	20.8	19.9	20.1
154.6	15.9	21.5	20.5	20.7

face coverage for this fine coal. The second series (Table 3) involved oil dosages that appear extremely heavy on a weight basis but are really more nearly the commercial dosages in view of the amount of surface exposed. The data of one table are not directly comparable with those of the other because of the temperature difference mentioned above. At the outset the untreated coals absorbed more oxygen than the oil-treated samples, but within a few days the oil-treated portions had surpassed the control samples. This effect is not pronounced for the lighter oil dosages, but is quite evident with the heavier treatments (Table 3).

Figure 2 shows the oxygen absorption by coal alone and that by coal given the heaviest oil treatment (columns 2 and 5, Table 3). It is plotted on a semilogarithmic scale to give details of the early days of the test and yet permit inclusion of the entire 155 days. The curves cross at approximately one week of exposure; it is perhaps unfortunate that the experiment was not continued for an additional 300 or more days, as extrapolation (if this is justifiable!) would seem to predict that at somewhat over a year's exposure the untreated coal would again be absorbing oxygen at the more rapid rate.

Oxygen Absorption by the Oil. Samples of oil, comparable in amount to that used in the heavy dosages shown above, were applied to -40 mesh silicon carbide and exposed to oxygen in the same apparatus. One sample was oil alone and the other was oil treated with a commercial antioxidant (Ethyl 720). The lower portion of Figure 3 shows that the antioxidant was essentially ineffective in preventing oxygen absorption. Both samples showed an induction period of about 7 days; the rate then increased rapidly for several days and subsequently leveled off.

Oxygen absorption curves for oil dispersed on expanded vermiculite showed no induction period, but had the general shape of those for coal (Fig. 1). Absorption rates for oil on vermiculite were higher than those for oil on silicon carbide, presumably because of greater surface area, but showed no appreciable change when antioxidants were added.

The existence of the induction period in the oxidation of the oil on silicon carbide may well be the key to the be-

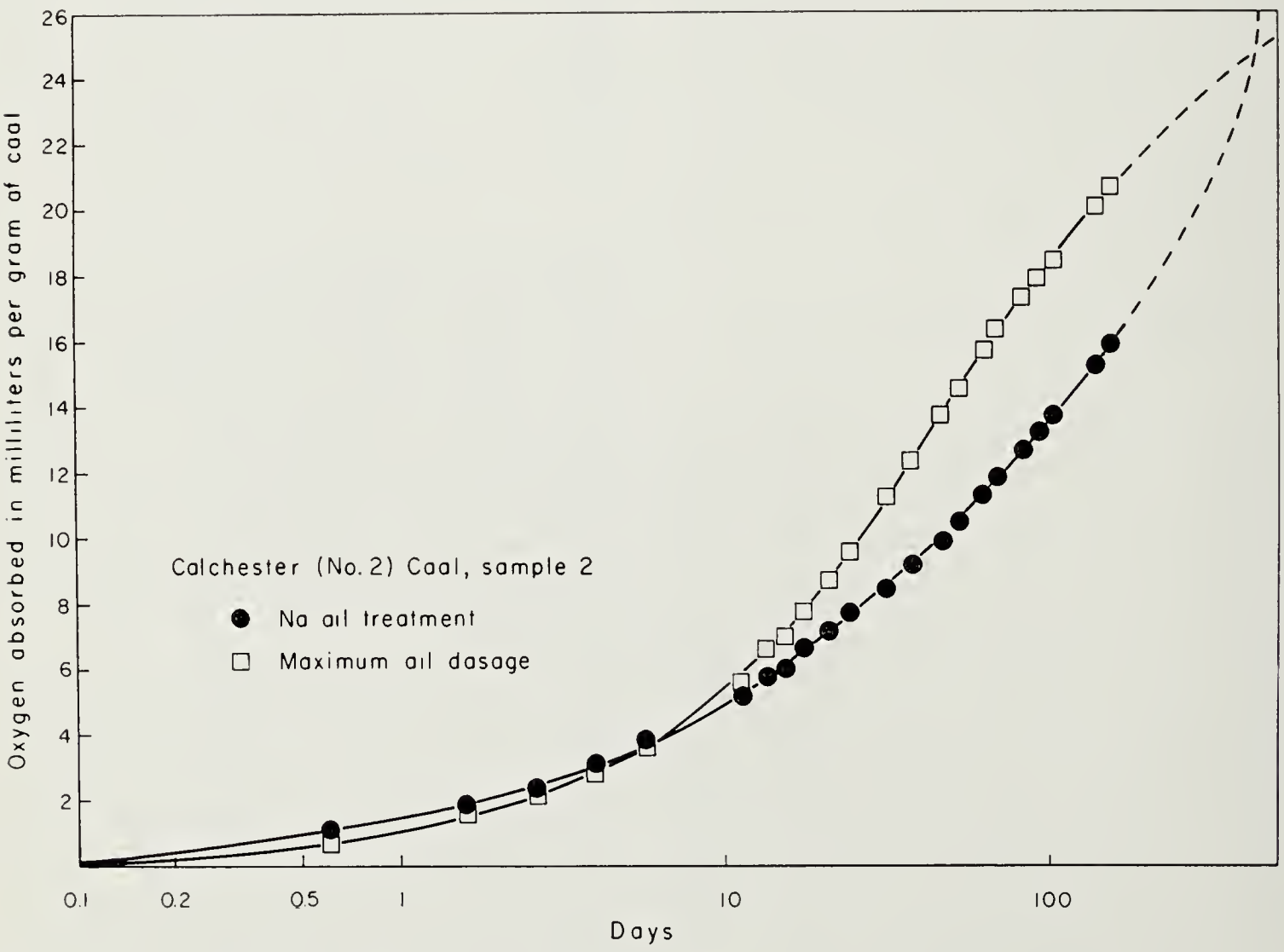

FIGURE 2. — Oxygen absorption by coal with and without oil treatment.

havior of the oil-treated coal samples shown in Table 3. The upper part of Figure 3 is a qualitative chart showing which oil-treated coal samples had absorbed the least and which had absorbed the most oxygen at given times. The time value at which maximum absorption moves from the untreated coal to the coal given the highest oil dosage coincides with the time of rapid increase in oxygen absorption by the oil alone, suggesting that at this point the oxidation of the oil is the important factor. After about 15 days of exposure, the untreated coal had absorbed less oxygen than any of the oil-treated samples in this series.

However, it appears that these rates are not additive. Figure 4 shows oxygen absorption of the oil alone calculated to milliliters of oxygen per 0.344 g of oil, plotted on the same graph as oxygen absorption per gram of untreated coal. The sum of these two is plotted as a third curve, and is lower than the curve showing the experimentally determined oxygen absorption by one gram of coal treated with this amount of oil. The oil appears to have a synergistic effect upon the oxidation of the coal.

This type of behavior has been observed in other systems. For example, Kern and Schnecko (1960) found that the rate of autoxidation of a mixture of methyl linoleate and dimethylbutadiene was higher than the sum of the rates of these compounds oxidized separately.

The curve of oil oxidation in Figure 3 is typical of the free radical chain mechanism of autoxidation, wherein oxidation is slow (induction period) until the concentration of free radicals becomes high enough to cause the oxidation velocity to increase at an increasing rate through the agency of chain-branching intermediates (Ingold, 1961). Conceivably, such radicals may play a part in the attack of oxidation-susceptible centers of the coal structure, with the result that the coal-oil mixture would absorb more oxygen in a given time than would be predicted by a simple addition of the separate rates.

It would not be justifiable to assume that all coal-oil mixtures would behave in this manner. In fact, Terpogosova (1954) reported that petroleum products protected coal from oxidation, Marma-

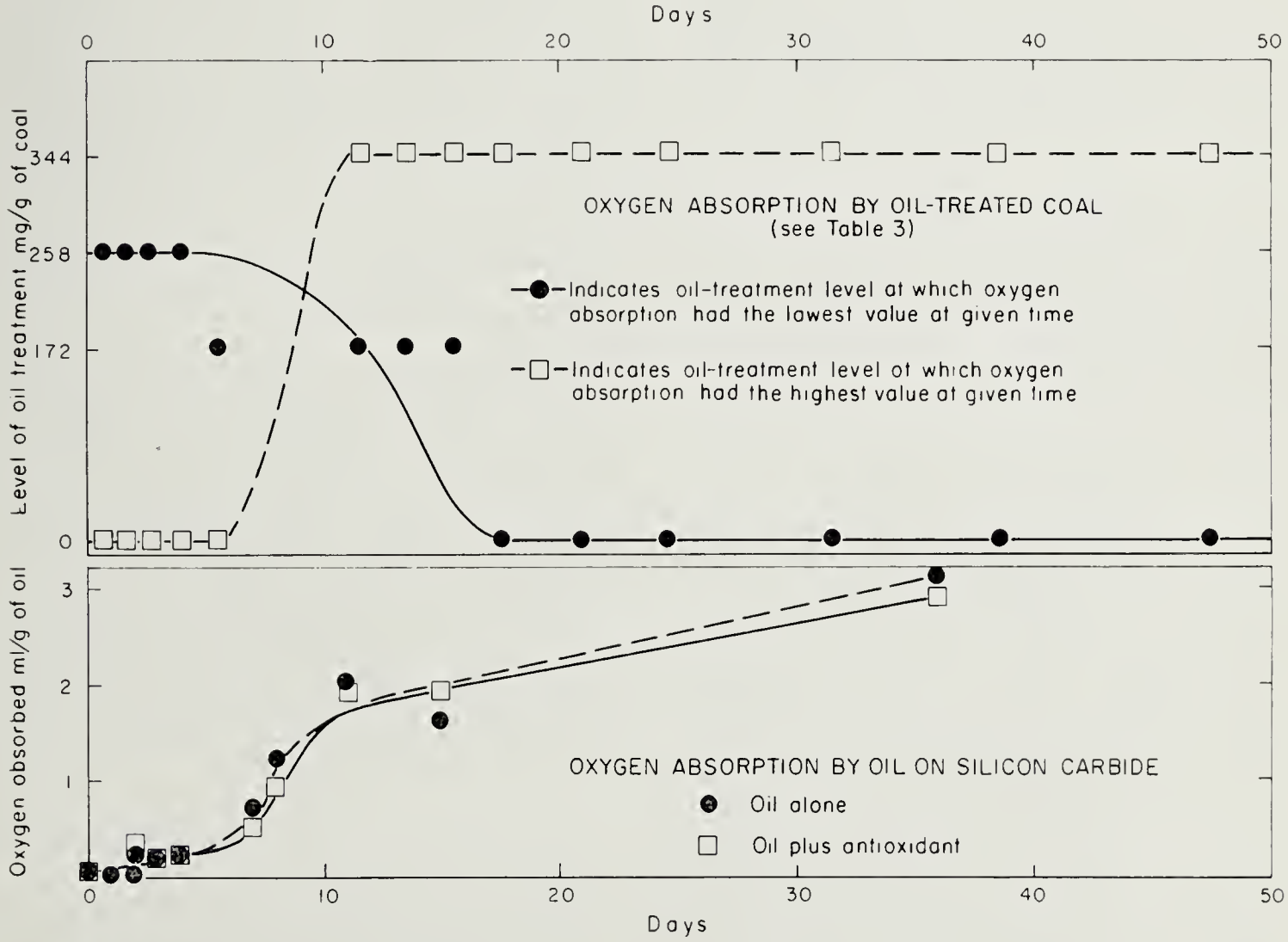

FIGURE 3. — Oxygen absorption by the oil (lower) compared with oil-treatment levels at which coal absorbed oxygen most rapidly and least rapidly (upper).

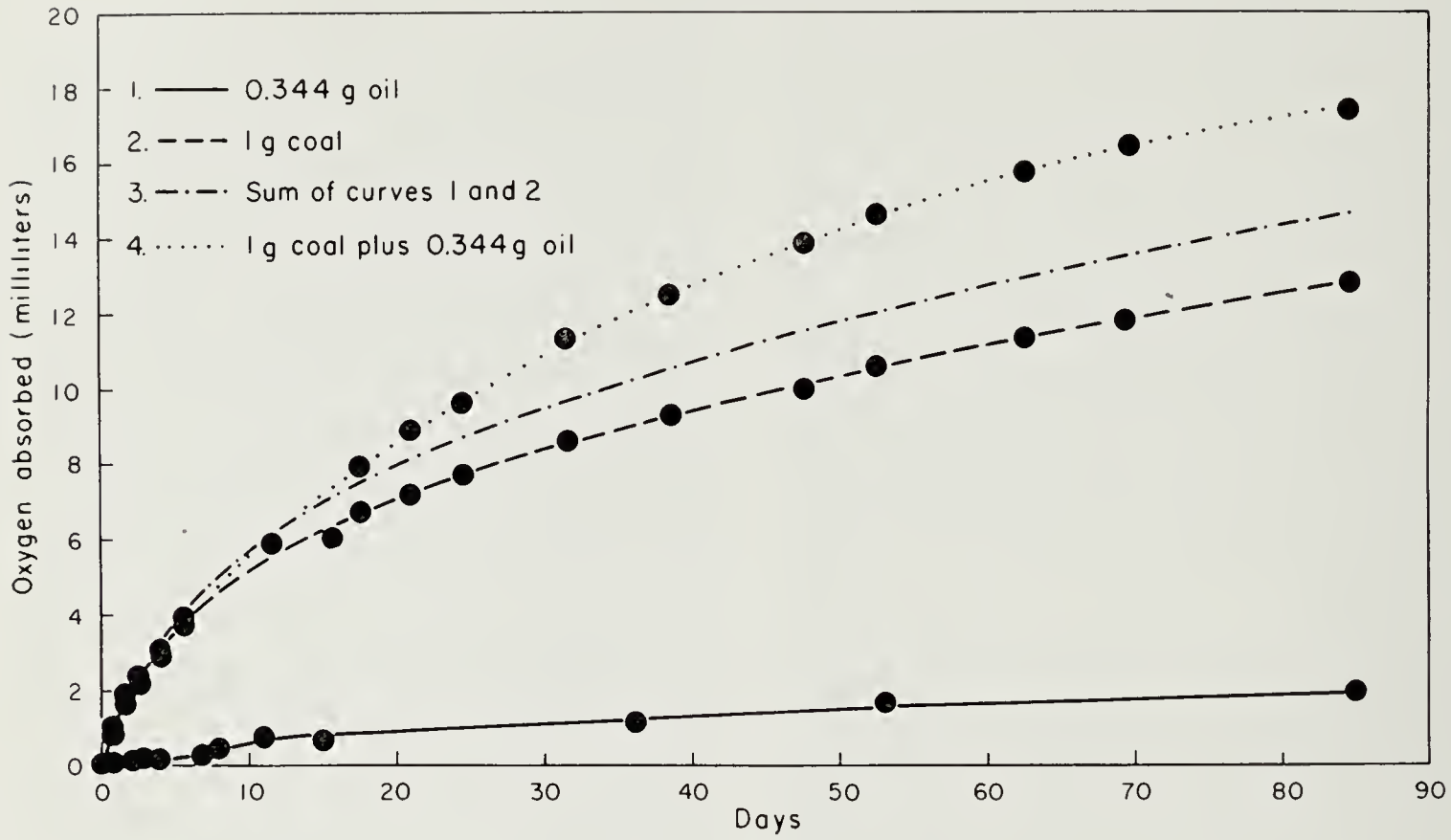

FIGURE 4. — Oxygen absorption by oil on silicon carbide and by Colchester (No. 2) Coal compared with that by oil-treated coal.

duke (1939) patented the use of a waxy coating for this purpose, and experiments run in the Illinois State Geological Survey laboratories (Yohe, Organist, and Lansford, 1952) showed that treating coal with paraffin wax lowered the rate of oxygen absorption.

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A COMPUTER PROGRAM FOR METEOROLOGIC DATA REDUCTION

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ABSTRACT. — A computer program is presented for use in reducing basic meteorologic data and presenting the data in tabular form. The program includes an adaptation of soil-moisture meter data to computer analysis.

Digital computers provide an efficient method for handling large amounts of data. The purpose of this paper is to present a computer program which reduces basic meteorologic data and presents them in summary tables. The program is used to calculate and tabulate meteorologic measurements in standard weather shelters, as well as additional measurements that are taken at or below the ground surface. A description of the overall research project and instruments used for meteorologic measurements has been published (Andersen and Levine, 1967). Parts of the computer program presented here could be applied directly to other projects which collect meteorologic data; with minor modifications it could be adapted to many more.

DESCRIPTION OF THE PROGRAM

The program is written in the Fortran II language (IBM, 1963) for the University of Illinois IBM 7094 computer. It requires 6,383 storage locations and approximately three minutes of computer time to process one year's data.

The program consists of a main section and one subroutine. The main program reads the input data, performs preliminary computations on the daily values, stores these daily values, and controls the use of subroutine MONTH. Subroutine MONTH performs monthly summary computations and produces the output in the form of monthly summary tables.

The input consists of up to 3 cards of data for each day. The number of cards varies with the season of the year since not all measurements can be made during freezing weather. Each of these cards has a number code to indicate which section of the main program is used for the preliminary data reduction. Figure 1 is an example of the data sheet which is used with this program. Each of the 3 daily cards has the day, month, year, and card number, followed by a maximum of 13 items of data, as identified in Figure 1. When there are missing data the columns are left blank, except that the temperature and dew duration columns are coded with the values of 999.0 and 99.0, respectively. Also, when the high range of the ammeter is used, a value of 9000 is added to the soil moisture basic data readings (microamperes).

One input data card, which is common to all runs, must precede the daily data cards. This card lists the number of days for the year on the last day of each month, e.g., 0, 31, 59, etc.

The output from the program is illustrated in Figures 2, 3, and 4. Some of the data are merely totaled and averaged for this presentation; other items

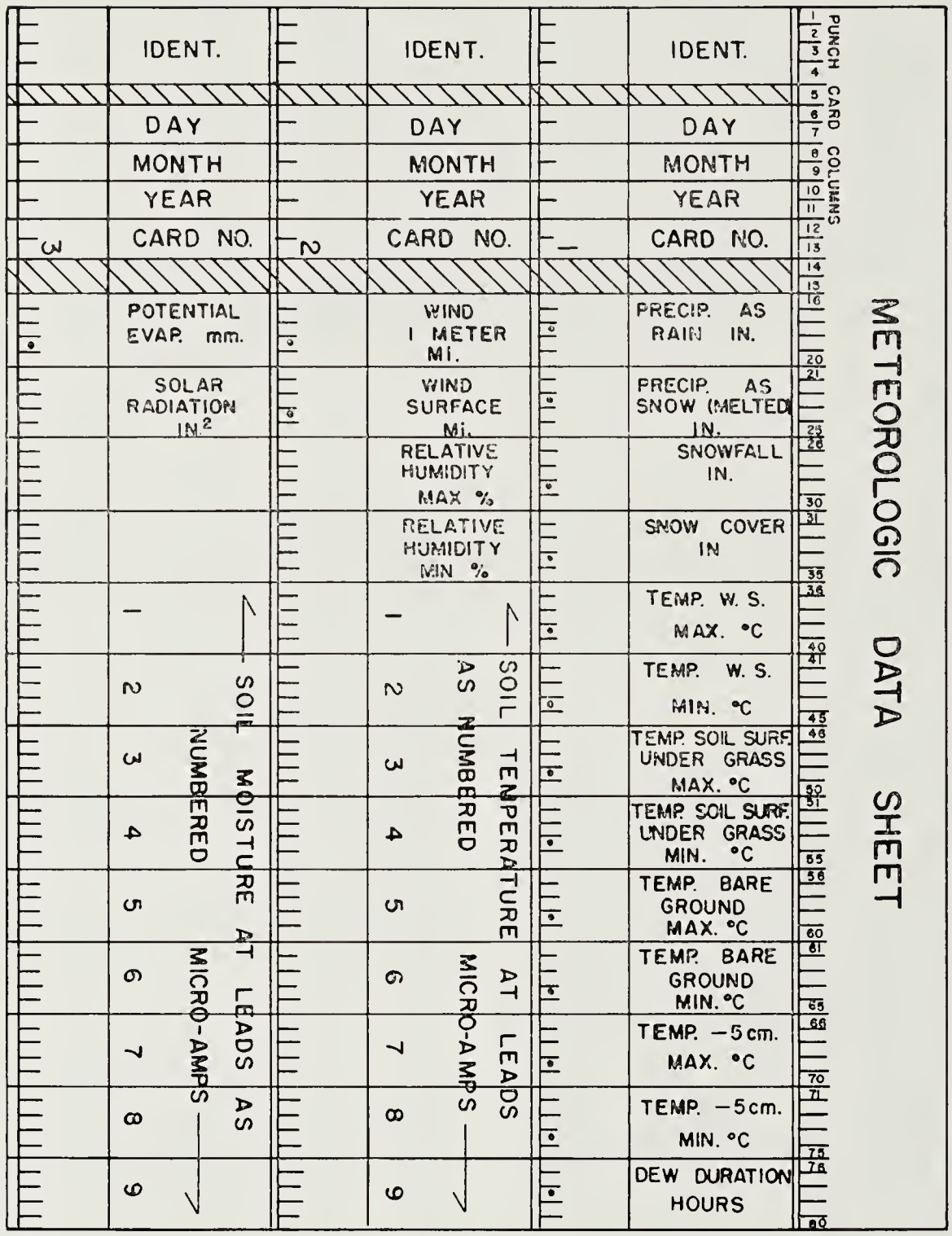

FIGURE 1. — Meteorologic data sheet.

require conversion of units from the raw data recorded on the meteorologic data sheet. Solar radiation, for example, is measured on a pyrheliograph and is converted from sq. in. of strip chart record to the appropriate units (Figure 2). The maximum and minimum temperatures for each day are averaged, and monthly means, maxima, and minima for each location are given.

The basic data for soil moisture and soil temperature are microampere readings obtained by using a Soiltest, Inc. soil moisture meter with soil moisture cell leads (wafers), and built-in thermistor units for soil temperature. The formulas used to convert these microampere data to soil temperature values and to corrected resistance values were obtained from the graphs given in the soil moisture meter manual (Soiltest, Inc., 1964) which is part of the testing kit. The equation with program statement numbers 250, 251, 303, and 350 were obtained by using a University of Illinois Computer Library Program for least square polynomial fit (DCS, UI, 1966) with points from the instruction manual graphs submitted as data. The equations for the straight line graphs in the instruction manual were computed manually and appear as statements 304 and 351. Statements 51 through 59 are the equations obtained from a least square polynomial fit of corrected resistance computations paired with gravimetric soil moisture measurements. These gravimetric measurements

MONTHLY SUMMARY OF METEOROLOGIC DATA, VET. MED. RESEARCH FARM, URBANA, ILLINOIS, MONTH 8 YEAR 1966

DATE	DAY OF YEAR	PRECIPITATION (MM) TOTAL PRECIP	PRECIP RAIN	PRECIP SNOW	SNOW FALL	SNOW COVER	DEW DURATION HR.	RELATIVE HUMIDITY WEATHER STATION MAX.	MIN.	WIND (KM) 1 METER	SURFACE	POTENTIAL EVAP MM	SOLAR RADIATION GMCAL/SQCM
1	213	10.41	10.41	.00	.00	.00	9.50	100	59	271.92	30.57	8.0	495.00
2	214	.00	.00	.00	.00	.00	10.50	94	64	210.78	64.36	10.2	576.00
3	215	.00	.00	.00	.00	.00	8.00	94	49	183.43	17.70	13.2	639.00
4	216	.00	.00	.00	.00	.00	11.50	81	34	20.92	3.22	.2	594.00
5	217	.00	.00	.00	.00	.00	9.75	97	80	24.13	1.61	14.4	495.00
6	218	.00	.00	.00	.00	.00	10.00	100	54	62.75	9.65	17.2	576.00
7	219	.51	.51	.00	.00	.00	13.25	100	65	157.68	17.70	18.2	450.00
8	220	3.56	3.56	.00	.00	.00	14.00	100	65	141.59	27.35	7.4	513.00
9	221	11.43	11.43	.00	.00	.00	9.00	100	61	104.58	14.48	8.4	549.00
10	222	17.02	17.02	.00	.00	.00	21.00	100	96	222.04	20.92	.0	126.00
11	223	.00	.00	.00	.00	.00	13.00	99	61	112.63	28.96	6.0	234.00
12	224	22.86	22.86	.00	.00	.00	6.00	100	56	125.50	1.61	11.1	585.00
13	225	3.05	3.05	.00	.00	.00	8.00	100	95	141.59	1.61	.2	171.00
14	226	1.27	1.27	.00	.00	.00	10.00	100	77	135.16	4.83	8.2	513.00
15	227	.51	.51	.00	.00	.00	14.00	100	77	217.21	22.53	8.8	450.00
16	228	.00	.00	.00	.00	.00	15.00	98	66	123.89	27.35	10.0	567.00
17	229	.00	.00	.00	.00	.00	13.50	98	61	96.54	3.22	7.4	405.00
18	230	7.11	7.11	.00	.00	.00	14.00	100	68	128.72	11.26	4.4	531.00
19	231	.00	.00	.00	.00	.00	7.00	99	72	152.85	4.83	6.0	459.00
20	232	.25	.25	.00	.00	.00	13.00	100	82	114.24	14.48	4.8	324.00
21	233	.51	.51	.00	.00	.00	13.00	100	76	244.57	35.40	7.0	432.00
22	234	.00	.00	.00	.00	.00	10.50	97	67	204.34	59.53	8.0	558.00
23	235	.00	.00	.00	.00	.00	11.50	95	58	168.94	49.88	7.6	585.00
24	236	.00	.00	.00	.00	.00	13.75	96	58	101.37	30.57	6.8	495.00
25	237	.00	.00	.00	.00	.00	12.75	96	58	93.32	30.57	8.0	468.00
26	238	.00	.00	.00	.00	.00	12.25	97	56	85.28	22.53	10.0	630.00
27	239	.00	.00	.00	.00	.00	12.50	98	60	75.62	12.87	8.4	549.00
28	240	.00	.00	.00	.00	.00	12.25	98	62	70.80	38.62	9.0	450.00
29	241	.00	.00	.00	.00	.00	13.25	98	64	48.27	9.65	12.6	450.00
30	242	.00	.00	.00	.00	.00	11.00	98	62	38.62	3.22	14.6	396.00
31	243	.00	.00	.00	.00	.00	12.50	98	61	43.44	1.61	16.0	855.00
TOTAL		78.49	78.49	.00	.00		365.25					272.1	15120.00
AVERAGE (TOTAL DAYS)		2.53	2.53	.00	.00	.00							
AVERAGE (RECORDED DAYS)							11.78	98	65	126.54	20.09	9.1	487.74

FIGURE 2. — Monthly summary of meteorologic data, page 1.

MONTHLY SUMMARY OF METEOROLOGIC DATA, VET. MED. RESEARCH FARM, URBANA, ILLINOIS, MONTH 8 YEAR 1966

TEMPERATURES (DEGREES CELSIUS)

DATE	DAY OF YEAR	WEATHER SHELTER			SOIL SURFACE UNDER 7-10 CM. GRASS			SOIL SURFACE BARE GROUND			5 CM. BENEATH SOIL SURFACE UNDER GRASS		
		MAX.	MIN.	MEAN	MAX.	MIN.	MEAN	MAX.	MIN.	MEAN	MAX.	MIN.	MEAN
1	213	30.0	18.9	24.4	39.0	24.0	31.5	52.0	20.5	36.2	30.0	25.0	27.5
2	214	21.7	9.4	15.5	31.0	18.0	24.5	41.0	11.5	26.2	28.0	20.0	24.0
3	215	22.2	9.4	15.8	36.0	18.5	27.2	50.0	12.0	31.0	29.5	20.5	25.0
4	216	24.4	10.0	17.2	37.5	18.5	28.0	53.0	13.0	33.0	30.0	21.0	25.5
5	217	26.1	11.1	18.6	40.0	19.5	29.7	56.0	14.0	35.0	31.0	22.0	26.5
6	218	27.2	11.7	19.4	40.0	20.0	30.0	56.0	15.5	35.7	31.0	22.5	26.7
7	219	27.8	13.3	20.5	38.0	20.0	29.0	51.0	15.0	33.0	29.5	22.0	25.7
8	220	28.3	14.4	21.3	41.0	21.0	31.0	57.0	16.0	36.5	31.0	23.0	27.0
9	221	25.6	14.4	20.0	37.0	21.0	29.0	53.0	17.0	35.0	31.0	22.5	26.7
10	222	17.8	13.3	15.5	25.0	20.0	22.5	25.0	15.0	20.0	24.0	21.5	22.7
11	223	22.8	10.6	16.7	32.0	19.0	25.5	36.0	13.0	24.5	27.0	21.0	24.0
12	224	24.4	10.6	17.5	35.0	22.5	28.7	50.0	19.0	34.5	28.0	23.0	25.5
13	225	17.8	15.0	16.4	24.0	22.0	23.0	23.0	20.0	21.5	24.0	23.0	23.5
14	226	26.7	17.8	22.2	35.0	25.0	30.0	48.0	22.0	35.0	29.0	25.0	27.0
15	227	28.3	18.3	23.3	35.0	24.5	29.7	46.0	21.0	33.5	29.5	25.0	27.2
16	228	25.6	11.1	18.3	35.0	20.0	27.5	49.0	15.0	32.0	29.0	22.0	25.5
17	229	26.7	11.1	18.9	37.0	21.5	29.2	51.0	17.0	34.0	30.0	23.0	26.5
18	230	29.4	13.9	21.6	39.0	24.0	31.5	54.0	20.0	37.0	31.0	25.5	28.2
19	231	25.0	15.6	20.3	34.0	23.0	28.5	45.0	19.0	32.0	31.0	24.5	27.7
20	232	23.3	16.1	19.7	30.0	24.0	27.0	36.0	21.0	28.5	28.0	25.0	26.5
21	233	28.9	17.8	23.3	35.0	24.0	29.5	48.0	21.0	34.5	30.0	25.0	27.5
22	234	22.8	7.8	15.3	33.0	18.0	25.5	43.0	11.0	27.0	27.5	20.0	23.7
23	235	20.6	7.2	13.9	32.0	16.0	24.0	43.0	10.5	26.7	25.5	19.0	22.2
24	236	21.7	7.2	14.4	32.0	15.5	23.7	43.0	10.5	26.7	25.0	18.5	21.7
25	237	22.2	7.8	15.0	35.0	16.0	25.5	47.0	11.5	29.2	26.0	19.0	22.5
26	238	26.1	8.9	17.5	33.0	18.0	25.5	50.5	14.5	32.5	28.5	18.5	23.5
27	239	27.8	12.2	20.0	35.5	20.0	27.7	51.5	15.5	33.5	29.0	21.5	25.2
28	240	27.8	12.2	20.0	35.0	20.0	27.5	50.5	15.5	33.0	29.5	21.5	25.5
29	241	27.8	13.9	20.8	35.5	20.5	28.0	50.5	16.0	33.2	29.5	22.0	25.7
30	242	27.8	14.4	21.1	35.0	22.0	28.5	54.0	17.5	35.7	30.0	23.0	26.5
31	243	28.3	14.4	21.3	35.0	22.0	28.5	53.0	17.5	35.2	30.5	23.0	26.7
MEAN		25.3	12.6	18.9	34.7	20.6	27.7	47.3	16.0	31.7	28.8	22.2	25.5
EXTREMES		30.0	7.2		41.0	15.5		57.0	10.5		31.0	18.5	

FIGURE 8. — Monthly summary of meteorologic data, page 2.

MONTHLY SUMMARY OF METEOROLOGIC DATA, VET. MED. RESEARCH FARM, URBANA, ILLINOIS, MONTH 8 YEAR 1966

SOIL MOISTURE AND SOIL TEMPERATURE (PERCENT AND DEGREES CELSIUS , 0800 C. M.)

DATE	DAY OF YEAR	CENTIMETERS BELOW SOIL SURFACE																	
		2.5		7.5		15.0		22.5		30.0		45.0		60.0		75.0		90.0	
		S M	S T	S M	S T	S M	S T	S M	S T	S M	S T	S M	S T	S M	S T	S M	S T	S M	S T
1	213	15.8	37.8	14.5	37.3	15.1	40.9	18.7	43.8	15.2	21.2	18.4	18.9	22.3	19.7	24.4	34.8	25.2	37.0
2	214	17.3	37.8	14.6	37.3	15.0	37.2	18.9	38.8	15.4	20.6	18.4	18.3	21.9	19.7	24.0	34.0	25.3	36.2
3	215	17.5	37.0	14.7	36.5	14.7	40.9	18.5	42.7	19.7	21.8	18.4	18.9	21.8	20.3	23.6	35.5	25.4	37.0
4	216	16.4	37.9	14.5	36.5	14.7	39.9	28.5	40.6	20.6	19.9	18.4	18.9	21.9	19.7	23.5	34.0	25.5	35.5
5	217	15.8	37.0	14.3	36.5	14.3	42.0	17.5	43.8	15.4	20.6	18.1	18.3	21.3	20.3	23.1	34.8	25.9	37.0
6	218	15.4	37.0	14.1	36.5	14.2	42.0	19.2	43.8	14.8	21.2	17.6	18.9	21.0	20.3	22.7	36.3	25.9	37.8
7	219	15.1	37.8	13.9	37.3	14.1	40.9	16.8	42.7	14.5	20.6	17.0	18.9	20.9	19.7	22.6	34.0	26.1	36.2
8	220	14.8	37.8	13.7	37.3	14.0	39.9	16.3	42.7	14.2	19.9	16.7	18.3	20.2	19.7	22.2	34.8	26.2	35.5
9	221	15.1	37.0	13.8	36.5	14.1	39.9	16.2	40.6	14.2	19.9	16.7	18.3	20.3	19.1	22.1	33.3	26.2	35.5
10	222	25.8	37.0	14.9	37.3	13.8	43.1	15.9	43.8	14.0	20.6	16.6	18.9	19.8	19.7	21.7	35.5	26.2	37.8
11	223	23.1	36.2	23.1	35.7	18.7	39.9	16.1	40.6	14.3	19.4	17.2	17.1	20.1	19.1	21.9	33.3	26.2	35.5
12	224	20.8	36.2	20.2	35.7	17.7	31.7	16.3	33.1	15.1	19.9	17.0	18.3	20.0	19.7	23.2	19.3	26.1	20.1
13	225	20.1	20.6	17.2	35.7	15.6	33.9	16.2	31.6	12.5	33.2	16.6	18.9	19.9	18.5	23.2	19.3	26.1	20.1
14	226	20.1	37.0	17.6	36.5	15.9	32.4	17.2	33.1	20.7	19.9	18.8	17.7	19.8	19.1	24.0	19.3	25.8	20.7
15	227	17.2	38.6	15.1	37.3	14.5	33.2	15.7	34.6	14.0	20.6	16.7	18.3	19.8	19.1	22.9	19.3	26.2	20.1
16	228	16.6	37.8	14.6	37.3	14.7	32.4	15.6	33.8	15.4	20.6	17.0	18.3	20.0	19.1	22.9	19.3	26.2	19.6
17	229	16.5	37.8	14.2	37.3	14.2	33.2	15.4	33.8	14.5	20.6	16.7	19.5	19.8	19.7	22.8	19.3	26.2	20.7
18	230	15.7	37.8	14.0	37.3	14.1	33.2	15.2	33.8	15.0	20.6	16.6	18.9	19.6	19.7	22.8	19.3	24.9	20.1
19	231	16.0	38.6	13.8	39.0	13.9	33.9	14.9	34.6	15.1	21.8	16.6	18.9	19.6	19.7	22.6	19.3	26.1	20.7
20	232	16.2	37.8	13.9	37.3	14.0	33.9	14.9	33.8	15.5	21.8	16.6	18.9	19.6	19.7	22.6	19.3	26.0	20.7
21	233	15.4	38.6	13.9	38.1	14.0	33.2	14.7	33.8	14.5	20.6	16.6	18.9	19.6	19.7	22.5	19.3	26.0	20.7
22	234	15.2	37.8	13.8	37.3	13.9	32.4	14.7	33.1	15.4	20.6	22.3	18.3	24.4	19.7	22.3	19.3	25.8	20.1
23	235	15.0	37.0	13.7	36.5	13.8	33.2	14.4	34.6	14.5	20.6	15.9	18.9	19.2	19.7	22.1	19.9	25.6	21.3
24	236	15.0	34.7	13.7	35.0	13.8	30.9	14.4	32.3	13.8	19.4	15.8	18.3	19.0	19.7	22.0	19.3	25.5	20.7
25	237	14.6	35.5	13.6	35.7	13.8	31.7	14.1	34.6	13.1	19.9	15.5	18.3	18.5	19.7	22.2	19.9	25.2	21.3
26	238	14.6	34.7	13.5	35.0	13.7	30.9	14.1	31.6	13.4	18.8	15.5	17.1	18.6	19.1	21.8	18.1	25.3	19.6
27	239	14.0	36.2	13.4	35.0	13.6	32.4	13.9	33.1	13.3	19.4	14.7	17.7	18.3	19.1	21.5	19.3	24.9	20.1
28	240	14.0	36.2	13.4	35.7	13.6	31.7	13.8	33.1	13.9	17.1	14.8	17.1	18.2	18.5	21.6	18.1	24.9	19.6
29	241	13.4	37.8	13.3	36.5	13.6	31.7	13.8	32.3	12.7	19.4	14.1	17.1	17.4	19.1	21.2	18.7	24.7	19.6
30	242	13.3	37.8	13.2	37.3	13.5	32.4	13.6	33.8	12.5	19.9	14.1	17.1	17.0	19.1	21.0	19.3	24.4	20.1
31	243	13.4	37.0	13.2	36.5	13.5	31.7	13.6	32.3	12.5	19.9	13.9	17.7	17.0	19.1	21.0	19.3	24.5	19.0
MEAN		16.4	36.6	14.7	36.7	14.5	35.4	16.1	36.5	14.8	20.6	16.8	18.3	19.9	19.5	22.5	24.7	25.6	26.0
MAXIMUM		25.8	38.6	23.1	39.0	18.7	43.1	28.5	43.8	20.7	33.2	22.3	19.5	24.4	20.3	24.4	36.3	26.2	37.8
MINIMUM		13.3	20.6	13.2	35.0	13.5	30.9	13.6	31.6	12.5	17.1	13.9	17.1	17.0	18.5	21.0	18.1	24.4	19.0

FIGURE 4. — Monthly summary of meteorologic data, page 3.

were obtained weekly during the summer months for each soil depth lead, ranging from 1 inch to 36 inches, over a period of two years, and were used to correlate the soil moisture resistance with the soil in which the cells were placed.

Anyone who uses the soil temperature-soil moisture portion of the program will find it necessary to revise 3 major items. The values of the coefficients for the 9 wafers are included in the program starting with statement number 247. These values vary with the wafers used. Statements 250, 303, and 350 describe the graph which is peculiar to each ammeter and may need to be changed if the curve supplied varies significantly from the formulas given here. The corrected resistance-soil moisture formulas, statements 51 through 59, need to be determined for the particular soil in which the cells are placed.

The calculation and tabulation of other data, such as wind, snow, humidity, and dew duration, are quite readily understood from the input data sheet and the output summary tables.

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Manuscript received May 23, 1968.

Appendix

The Program:

```
$       FASTRAN
$       GO
C       METEOROLOGIC DATA PROCESSING PROGRAM ,   C Z R PROJECT LED BY
C       FERRON L ANDERSEN , PROGRAMMER- J.KENT MITCHELL   6/8/65
C       THE FOLLOWING SECTION IS THE MAIN PROGRAM WHICH READS DATA,
C       COMPUTES CARD DATA, AND CONTROLS THE MONTH SUBROUTINE
        DIMENSION  A(13),C1(40,12),C2(40,12),CDY(40),LDAY(13),LEAP(13),C1T
       1(12),C4(40,18),COEE(10),IST(10),DN1(12),DN2(12),C2T(12),CT(12),ST(
       210),SM(10),C4T(18),C4N(18),C4X(18)
        COMMON A,KDY,KMO,KYR,KDYZ,KMOZ,KYRZ,C1,C2,CDY,LDAY,LEAP,C1T,C4,COE
       1E,IST,DN1,DN2,C2T,CT,C4T,C4N,C4X
        RIT 7,90,LDAY,LEAP
   90 FORMAT (26I3)
        KDYT=1
        KMOT=12
        KYRT=35
        KDYZ=1
        KMOZ=12
        KYRZ=35
        DO 1860 K=1,12
        DN1(K)=0.0
        DN2(K)=0.0
        C2T(K)=0.0
 1860 C1T(K)=0.0
        DO 3860 J=1,40
        DO 3861 K=1,12
        C1(J,K)=0.0
        C2(J,K)=0.0
 3861 CONTINUE
        DO 3860 K=1,18
        C4(J,K)=0.0
        C4T(K)=0.0
        C4X(K)=-100.0
        C4N(K)=100.0
 3860 CONTINUE
        DO 9 J=1,10,3
    9 CT(J)=-100.0
        DO 10 J=2,11,3
   10 CT(J)=100.0
    1 RIT 7,50,KDY,KMO,KYR,NC,(A(N),N=1,13)
   50 FORMAT (5X,4I2,2X,13F5.0)
    4 IF (KMO-KMOT)5,6,5
    5 CALL MONTH
        KMOT=KMO
    6 KMOZ=KMO
        KYRZ=KYR
        GO TO (100,200,300,400),NC
  400 GO TO 1
C       THIS SECTION PROCESSES THE DATA ON CARD 1
C           THE FOLLOWING COMPUTES THE PRECIPITATION DATA
  100 C1(KDY,1)=(A(1)+A(2))*25.4
```

```
      C1(KDY,2)=A(1)*25.4
      C1(KDY,3)=A(2)*25.4
      C1(KDY,4)=A(3)*25.4
C     THE FOLLOWING COMPUTES SNOW COVER DATA
      C1(KDY,5)=A(4)*25.4
C         THE FOLLOWING COMPUTES TEMPERATURE DATA
      C2(KDY,1)=A(5)
      C2(KDY,2)=A(6)
      C2(KDY,3)=(A(5)+A(6))/2.0
      C2(KDY,4)=A(7)
      C2(KDY,5)=A(8)
      C2(KDY,6)=(A(7)+A(8))/2.0
      C2(KDY,7)=A(9)
      C2(KDY,8)=A(10)
      C2(KDY,9)=(A(9)+A(10))/2.0
      C2(KDY,10)=A(11)
      C2(KDY,11)=A(12)
      C2(KDY,12)=(A(11)+A(12))/2.0
C         THE FOLLOWING LISTS THE DEW DURATION DATA
      C1(KDY,6)=A(13)
      GO TO 1
C         THIS SECTION PROCESSES THE DATA ON CARD 2
C         THE FOLLOWING COMPUTES THE WIND DATA
  200 C1(KDY,9)=A(1)*1.609
      C1(KDY,10)=A(2)*1.609
C         THE FOLLOWING LISTS THE RELATIVE HUMIDITY DATA
      C1(KDY,7)=A(3)
      C1(KDY,8)=A(4)
C         THE FOLLOWING COMPUTES THE SOIL TEMPERATURE DATA
  247 COEE(1)=0.95
      COEE(2)=0.97
      COEE(3)=1.08
      COEE(4)=1.05
      COEE(5)=0.98
      COEE(6)=1.03
      COEE(7)=0.99
      COEE(8)=1.01
      COEE(9)=0.92
      DO 230 M=1,9
      X=COEE(M)
      Y=ELOG(X)
      COEE(M)=Y/2.3025851
  230 CONTINUE
      N=4
      DO 204 M=1,9
      N=N+1
      IF (A(N))284,284,220
  284 I=M*2
      C4(KDY,I)=A(N)
      IST(M)=2
      GO TO 204
  220 X=A(N)/100.0
  250 Y=5.198222-5.3602392*X+8.4944908*X**2+1.5319932*X**3-30.141324*X**
```

```
     14+48.404556*X**5-35.559542*X**6+12.763019*X**7-1.8107367*X**8
      Y=Y+COEE(M)
  251 V =6.6425621 -3.8361934 *Y +0.99996156 *Y **2 -0.15570077 *Y **3
     1 +0.010272893 *Y **4
      ST(M)=((V*100.0-32.0)*5.0)/9.0
      I=M*2
      C4(KDY,I)=ST(M)
      IST(M)=1
  204 CONTINUE
      GO TO 1
C          THIS SECTION PROCESSES THE DATA ON CARD 3
C          THE FOLLOWING COMPUTES THE EVAPORATION AND SOLAR RADIATION
C     DATA
  300 C1(KDY,11)=A(1)
      C1(KDY,12)=A(2)*900.0
C          THE FOLLOWING COMPUTES THE SOIL MOISTURE DATA
      N=4
      DO 301 M=1,9
      N=N+1
      IF (A(N))320,320,321
  320 I=M*2-1
      C4(KDY,I)=A(N)
      GO TO 301
  321 LL=IST(M)
      GO TO (5321,5322),LL
 5322 I=M*2-1
      C4(KDY,I)=A(N)
      GO TO 301
 5321 X=A(N)/100.0
      IF (X-90.0)303,302,302
  303 Y=5.198222-5.3602392*X+8.4944908*X**2+1.5319932*X**3-30.141324*X**
     14+48.404556*X**5-35.559542*X**6+12.763019*X**7-1.8107367*X**8
      GO TO 304
  302 X=X-90.0
  350 Y=6.2300594-6.3183331*X+14.141023*X**2-18.53311*X**3+12.955208*X**
     14-4.5519551*X**5+0.63068734*X**6
  304 Z=19.6672-2.8096*Y
      TFA=ST(M)*1.8+32.0
  351 V =7.0 +0.01428 *(TFA -60.0) -(0.3565 +0.0008 *(TFA -60.0)) *Z
      GO TO (51,52,53,54,55,56,57,58,59),M
   51 SM(M)= 152.32435 -100.18720*V +26.117317 *V**2 -2.3733765 *V**3
      GO TO 22
   52 SM(M)= 66.341936 -22.163397*V +2.7940253 *V**2 -0.11024337 *V**3
      GO TO 22
   53 SM(M)= 93.889043 -44.061439*V +7.9730356 *V**2 -0.47993947 *V**3
      GO TO 22
   54 SM(M)= 88.216981 -44.752830*V +9.7633048 *V**2 -0.79095782 *V**3
      GO TO 22
   55 SM(M)= 111.46129 -73.612518*V +21.859611 *V**2 -2.2701934 *V**3
      GO TO 22
   56 SM(M)= 121.4559  -92.47035 *V +30.059332 *V**2 -3.3267269 *V**3
      GO TO 22
   57 SM(M)= 139.46608 -110.22338*V +35.539901*V**2 -3.8253506*V**3
```

```
      GO TO 22
   58 SM(M)= 11.931366 +11.093733*V -1.8405329 *V**2 -0.057838565 *V**3
      GO TO 22
   59 SM(M)= 228.89604 -191.243 *V  +59.019853 *V**2 -5.9828745 *V**3
   22 I=M*2-1
      C4(KDY,I)=SM(M)
  301 CONTINUE
      GO TO 1
      END
```

```
$       FASTRAN
$       GO
        SUBROUTINE MONTH
C           THIS SUBROUTINE COMPUTES THE MONTHLY AVERAGES AND OUTPUTS THE
C       MONTHLY SUMMARY
        DIMENSION  A(13),C1(40,12),C2(40,12),CDY(40),LDAY(13),LEAP(13),C1T
       1(12),C4(40,18),COEE(10),IST(10),DN1(12),DN2(12),C2T(12),CT(12),ST(
       210),SM(10),C4T(18),C4N(18),C4X(18)
        COMMON A,KDY,KMO,KYR,KDYZ,KMOZ,KYRZ,C1,C2,CDY,LDAY,LEAP,C1T,C4,COE
       1E,IST,DN1,DN2,C2T,CT,C4T,C4N,C4X
        LYR=KYRZ
 1705   IF (LYR)1702,1703,1704
 1704   LYR=LYR-4
        GO TO 1705
 1702   KB1=LDAY(KMOZ)
        KB2=LDAY(KMOZ+1)
        N=KB2-KB1
        XN=N
        GO TO 1706
 1703   KB1=LEAP(KMOZ)
        KB2=LEAP(KMOZ+1)
        N=KB2-KB1
        XN=N
 1706   WOT 6,1750,KMOZ,KYRZ
 1750   FORMAT (1H1/////   20X,
       1 54H     MONTHLY SUMMARY OF METEOROLOGIC DATA, VET. MED. R,
       2 38HESEARCH FARM, URBANA, ILLINOIS, MONTH ,I2, 9H  YEAR 19,I2//)
        WOT 6,2750
 2750   FORMAT (10X,    10H----------,
       4 54H--------------- - ------   ------- ------ -     -----,
       5 56H--------------- - ------   ------- ------ -     -------/
       6            10X,10H       DAY,
       7 54H              PRECIPITATION (MM)                  DEW  ,
       8 54H RELATIVE HUMIDITY       WIND       POTENTIAL   SOLAR )
        WOT 6,1770
 1770   FORMAT (10X,    10H DATE  OF ,
       1 54H -------------- --------   ------- -------- DURATION ,
       2 56H  WEATHER STATION         (KM)          EVAP    RADIATION /
       3            10X,10H      YEAR,
       4  53H   TOTAL   PRECIP   PRECIP    SNOW      SNOW          ,
       5 54H  ---------------  ----------------                    /
       6            10X,          10X,
       7  53H  PRECIP    RAIN     SNOW     FALL    COVER       HR.,
       8  56H    MAX.     MIN.   1 METER  SURFACE     MM   GMCAL/SQCM)
        WOT 6,1771
 1771   FORMAT (10X,    10H ---- ----,
       1 54H -------  -------  -------  -------  -------  - -----  ,
       2 56H -------  -------  -------  -------  -----  -  - -------  //)
        DO 1707 J=1,N
        CDY(J)=KB1+J
        IF (C1(J,6)-99.0)1901,1900,1900
 1900   C1(J,6)=-0.0
        GO TO 1902
```

```
1901 DN1(6)=DN1(6)+1.0
1902 DO 1708 K=1,5
     IF (C1(J,K))1709,1709,1708
1709 C1(J,K)=0.0
1708 CONTINUE
     DO 2708 K=1,12
2708 C1T(K)=C1T(K)+C1(J,K)
     WOT 6,1751,J,CDY(J),(C1(J,K),K=1,12)
1751 FORMAT ( 9X,I5,F6.0,F8.2,5F9.2,2F9.0,2F9.2,F9.1,F10.2)
     DO 1707  L=7,12
     IF (C1(J,L))4801,4801,4802
4801 C1(J,L)=0.0
     GO TO 1707
4802 DN1(L)=DN1(L)+1.0
1707 CONTINUE
     WOT 6,1752,(C1T(L),L=1,4),C1T(6),C1T(11),C1T(12)
1752 FORMAT (1H0,13X,5HTOTAL,4F9.2,9X,F9.2,36X,F9.1,F10.2)
     DO 1800 L=1,5
     C1T(L)=C1T(L)/XN
1800 CONTINUE
     DO 4820  L=6,12
     IF (DN1(L))4810,4810,4811
4810 C1T(L)=0.0
     GO TO 4820
4811 C1T(L)= C1T(L)/DN1(L)
4820 CONTINUE
     WOT 6,1753,(C1T(L),L=1,12)
1753 FORMAT (1H0,11X,7HAVERAGE,5F9.2/                                  9X,12H(TOTA
    1L DAYS)//12X,7HAVERAGE,45X,F9.2,2F9.0,2F9.2,F9.1,F10.2/9X,15H(RECO
    2RDED DAYS))
     WOT 6,1750,KMOZ,KYRZ
     WOT 6,1754
1754 FORMAT (57X,
    2      33HTEMPERATURES (DEGREES CELSIUS)   /10X,118H---------- ----
    3--------    -------- - ------  -------- -- --- -     ----- *- ----
    4--------  - --------- - ------  ------/
    5             10X,10H       DAY,
    6 54H      WEATHER SHELTER                                SOIL S,
    7 54HURFACE                              5 CM. BENEATH SOIL    )
     WOT 6,1780
1780 FORMAT (    10X,10H DATE  OF ,
    1 54H                                  UNDER 7-10 CM. GRASS   ,
    2 54H         BARE GROUND              SURFACE UNDER GRASS    /
    3             10X,10H      YEAR,
    4 54H --------------- - -------  ------- -- --- -     ---- ,
    5 54H --------------- - -------  ------- ------ -     ---- /
    6             10X,           10X,
    7  53H    MAX.     MIN.     MEAN     MAX.     MIN.     MEAN,
    8  53H    MAX.     MIN.     MEAN     MAX.     MIN.     MEAN)
     WOT 6,1781
1781 FORMAT (    10X,10H ---- ----,
    1 54H -------  -------  -------  -------  -------  - ----- ,
    2 54H -------  -------  -------  -------  -------  - ----- //)
```

```
      DO 1710 J=1,N
      DO 1713 K=1,12
      IF (C2(J,K)-999.0)1711,1712,1712
1712  C2(J,K)=0.0
      GO TO 1714
1711  DN2(K)=DN2(K)+1.0
1714  C2T(K)=C2T(K)+C2(J,K)
1713  CONTINUE
      WOT 6,1755,J,CDY(J),(C2(J,K),K=1,12)
1755  FORMAT (9X,I5,F6.0,F8.1,11F9.1)
      DO 109 K=1,10,3
      IF (C2(J,K)-CT(K))109,109,108
 108  CT(K)=C2(J,K)
 109  CONTINUE
      DO 111 K=2,11,3
      IF (C2(J,K)-CT(K))110,111,111
 110  CT(K)=C2(J,K)
 111  CONTINUE
1710  CONTINUE
      DO  4830 K=1,12
      IF (DN2(K))4831,4831,4832
4831  C2T(K)=0.0
      GO TO 4830
4832  C2T(K)=C2T(K)/DN2(K)
4830  CONTINUE
      WOT 6,1756,(C2T(K),K=1,12),CT(1),CT(2),CT(4),CT(5),CT(7),CT(8),
     1CT(10),CT(11)
1756  FORMAT (1H0,14X,4HMEAN,12F9.1//11X,8HEXTREMES,4(2F9.1,9X))
      WOT 6,1750,KMOZ,KYRZ
      WOT 6,3004
3004  FORMAT  (30X,80HSOIL MOISTURE AND SOIL TEMPERATURE  (PERCENT AND D
     1EGREES CELSIUS  ,  0800 A. M.)/  10X,   118H---------- ----------
     2------ -     -----   - --- -    ---- -- -- ---       --- -  -  ---
     3--------  - ----------------/ 10X,     10H       DAY,
     4 60H                                  CENTIMETERS BELOW SOIL SU,
     5 48HRFACE                                          /
     6 10X, 10H DATE  OF ,
     7 60H     2.5          7.5         15.0         22.5         30.0    ,
     8 48H    45.0         60.0         75.0         90.0     )
      WOT 6,3005
3005  FORMAT (10X,    10H      YEAR,
     1 60H ---------- --- - -----   --------  ----- -     ------*- --,
     2 48H ---------- --- ------- - --------- ----- -    /
     3  20X,
     4 60H   S M   S T   S M   S T   S M   S T   S M   S T   S M   S T,
     5 48H   S M   S T   S M   S T   S M   S T   S M   S T/
     6 10X, 10H ---- ----,
     7 60H ----- ----- ----- -----   --- ----- ----- - -   ----- -- --,
     8 48H ----- ----- ----- -----   --- ----- ----- - -  //)
      DO 3006 J=1,N
      DO 4100 K=1,18
      C4T(K)=C4T(K)+C4(J,K)
4100  CONTINUE
```

```
      WOT 6,3007,J,CDY(J),(C4(J,K),K=1,18)
3007 FORMAT (9X,I5,F6.0,18F6.1)
3006 CONTINUE
      DO 4103 K=1,18
      C4T(K)=C4T(K)/XN
4103 CONTINUE
      WOT 6,4600,(C4T(K),K=1,18)
4600 FORMAT (1H0,15X,4HMEAN,18F6.1)
      DO 4104 K=1,18
      DO 4104 J=1,N
      IF (C4X(K)-C4(J,K))4150,4150,4151
4150 C4X(K)=C4(J,K)
4151 IF (C4N(K)-C4(J,K))4104,4104,4153
4153 C4N(K)=C4(J,K)
4104 CONTINUE
      WOT 6,4601,(C4X(K),K=1,18)
4601 FORMAT (1H0,12X,7HMAXIMUM,18F6.1)
      WOT 6,4602,(C4N(K),K=1,18)
4602 FORMAT (1H0,12X,7HMINIMUM,18F6.1)
      DO 4105 K=1,18
      C4T(K)=0.0
      C4N(K)=100.0
      C4X(K)=-100.0
4105 CONTINUE
      DO 1850 K=1,12
      DN1(K)=0.0
      DN2(K)=0.0
      C2T(K)=0.0
1850 C1T(K)=0.0
      DO 742 J=1,10,3
 742 CT(J)=-100.0
      DO 743 J=2,11,3
743 CT(J)=100.0
      RETURN
      END
```

GEOGRAPHICAL APPROACHES TO 7TH APPROXIMATION SUBGROUP ANALYSIS OF SELECTED SOIL INTERPRETATION MAPS

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Abstract. — The potential value of the United States' new soil classification system, the 7th Approximation, is investigated with respect to its applicability in soil limitation problems. Soil limitation studies for the construction of small buildings are made by utilizing detailed soil data and more general soil data organized within the framework of the 7th Approximation subgroup. The results of the research show that 7th Approximation soil limitation analysis can provide a versatile and easy method of developing land use studies without unduly sacrificing the accuracy attained by utilizing detailed soil data.

The theoretical framework of the 7th Approximation (Soil Survey Staff, 1960) is presented in a form that permits the development of maps and the testing of the applicability of the new soil classification system to soil-oriented problems. Research is needed to explore the potential applications of the 7th Approximation beyond its known and theoretical value.

A modified generalized 7th Approximation soil map (Klink, 1967, p. 109) of a county in east-central Illinois is constructed in an effort to ascertain the value of 7th Approximation subgroups in developing a soil use interpretation map for the construction of cottages and utility buildings. A comparison between the results obtained from a detailed soil analysis using soil type-slope-erosion data and the more general 7th Approximation subgroups is made in order to validate or invalidate the 7th Approximation application. Selected geographical interrelationships between the subgroup soil regions and the soil interpretation map for the construction of cottages and utility buildings are analyzed.

The United States Soil Conservation Service has developed over 70 different interpretations of soil suitability or limitation for various land use purposes (Soil Conservation Service Staff, 1966). Soil productivity and drainage characteristic maps are the most common in use; however, the relationships between soil and recreation, surface transportation, construction, industrial location, constructional materials, etc. are becoming increasingly important to many groups of researchers. Planners, soil scientists, engineers, geographers, rural developers, and recreational experts all need and contribute to an understanding of the interrelationships between characteristics of the soil and phenomena related to its best use within a given set of condi-

tions. Only through an understanding of these interrelationships can a proper utilization of land be achieved. Many unicriterial soil interpretation maps judge soil limitation as (1) slight or no limitation (2) moderate limitation (3) severe limitation with respect to the land use under study. In the construction of a limitation map a soil series can be subdivided into textural and then slope-erosion classes in order to obtain a very detailed study unit. Each detailed study unit has a slight, moderate or severe limitation with respect to the phenomena under study. For example in a soil interpretation map for the construction of cottages and utility buildings (with septic tank) each soil type with a different slope-erosion class is ranked severe, moderate or slight limitation for its suitability in this type of construction (Soil Conservation Service Staff, 1966). The soil factors considered in judging soil suitability in the construction of cottages and utility buildings are wetness, permeability, texture, flood hazard, slope, rockiness-stoniness and depth to bedrock. All the available detailed soil data are converted into the proper limitation class and a composite map is made for the desired area. The resulting regions on the map show (1) areas with the greatest suitability for construction of cottages and utility buildings, (2) areas where precautions must be taken before commencement of construction, (3) areas where it is unwise to construct the buildings.

The development of a limitation map based on detailed soil information is time consuming. The accuracy of soil data is excellent; however, conversion of the data to fit general planning needs over a wide range of conditions gives a subjective accuracy to the map. Undoubtedly, the severe, moderate, and slight limitation regions on the resulting map are accurate within the framework of the study since they are derived from very detailed soil series slope-erosion data. It seems feasible to use higher levels of soil classification in order to develop broadly defined soil limitation regions of selected phenomena. A more general soil data source, if properly organized for a particular study, may serve in drawing useable soil interpretation maps which approach in accuracy similar maps based on highly detailed studies. In many studies, development of a map derived from higher levels of soil classification ultimately makes possible a more rapid use and interpretation of many land use features without sacrificing the required degree of accuracy. A 7th Approximation subgroup limitation map of a county can be constructed in a fraction of the time required in the construction of a soil type limitation map provided the subgroup map and average limitation values for subgroups are available. Thus a substantial saving of time is possible if the 7th Approximation limitation studies provide the accuracy demanded in its use.

Subgroups are subdivisions of great groups which are defined largely by the presence or absence of diagnostic horizons and the arrangement of the horizons. Common differentiae of diagnostic horizons include illuviation of clay, iron and humus; thick, dark-colored surface horizons; pans that interfere with root development, water movement or both; and hori-

zons formed under cultivation. (Soil Survey Staff, 1960, p. 14)

The soil properties considered in the determination of the degree of limitation for the construction of cottages are not the specific soil properties used to define subgroups. It is not an objective of this paper to prove that the defined properties of the subgroups determine or explain the properties considered significant in soil limitation for the construction of cottages. Undoubtedly the defined subgroup soil properties reflect characteristics not specifically stated in a subgroup definition. It is the soil scientist that can best analyze the kind and degree of relationship that exists between the defined properties of subgroups and other soil properties within subgroups. An objective of the paper is to determine if a suborder map can present soil patterns that are of value to a geographer in the interpretation of a land limitation study.

If a subgroup map proves to be of value in predicting a soil limitation for cottage construction, then the reasons for the apparent success should be analyzed by the soil scientist, not the geographer, in order to determine whether there is a theoretical basis which will enable expanded use of the subgroup in selected land use studies.

The paper asks the question: Can a geographer or planner use a subgroup map for any planning purpose? The answer to this question cannot always be theoretically determined from subgroup soil property definitions; therefore, an application of a subgroup map may shed light on the possibility of its use in selected aspects of land use study.

Procedure

A 1964 7th Approximation subgroup map of soil distribution in Coles County, Illinois is constructed (see Figure 1) using Conservation Needs Inventory 2% sample data (Conservation Needs Inventory, 1957). Each major soil subgroup in Coles County, Illinois has its degree of limitation with respect to cottage and utility buildings with septic tank calculated. The group of soils that in total comprise a subgroup of the 7th Approximation in Coles County are analyzed and an average limitation value is calculated. Table 1 shows the values assigned to the degree of limitation in this study.

If a 7th Approximation subgroup has combined soils of very similar reaction with respect to the phenomena under study then the differential distributional character of the soils within the subgroup is of little significance. If, on the other hand, the subgroup combines soils of widely varying reaction with respect to the phenomena under study, then use of the 7th Approximation subgroup will yield results that are subject to great error.

Subgroup limitation values used in conjunction with a version of Figure 1 results in a soil interpretation map, Figure 2, which is a prediction of the degree of limitation in the construction of cottages and utility buildings. It might be noted from Figure 2 that no part of Coles County is free from moderate to severe limitations with respect to use for construction cottages and utility buildings since all areas (when generalized) fall into categories 3 to 5. Closer scrutiny, however, reveals that a large portion of the county suffers from only moderate limitation, there being only restricted areas in categories above 4. Limitations under 3 are found in the study area but do not appear in a generalized map as shown in Figure 2. Figure 2 is generalized for purposes of clarity but a more detailed version can be prepared for actual land use planning if the map is proved accurate for its required use.

The degree of accuracy of Figure 2 is ascertained through the construction and analysis of a map (Figure 3) which compares the 7th Approximation subgroup limitation map with one based on the most detailed soil information available (soil type-slope-erosion classes). The comparative study uses the same 2% sample data but the organization is

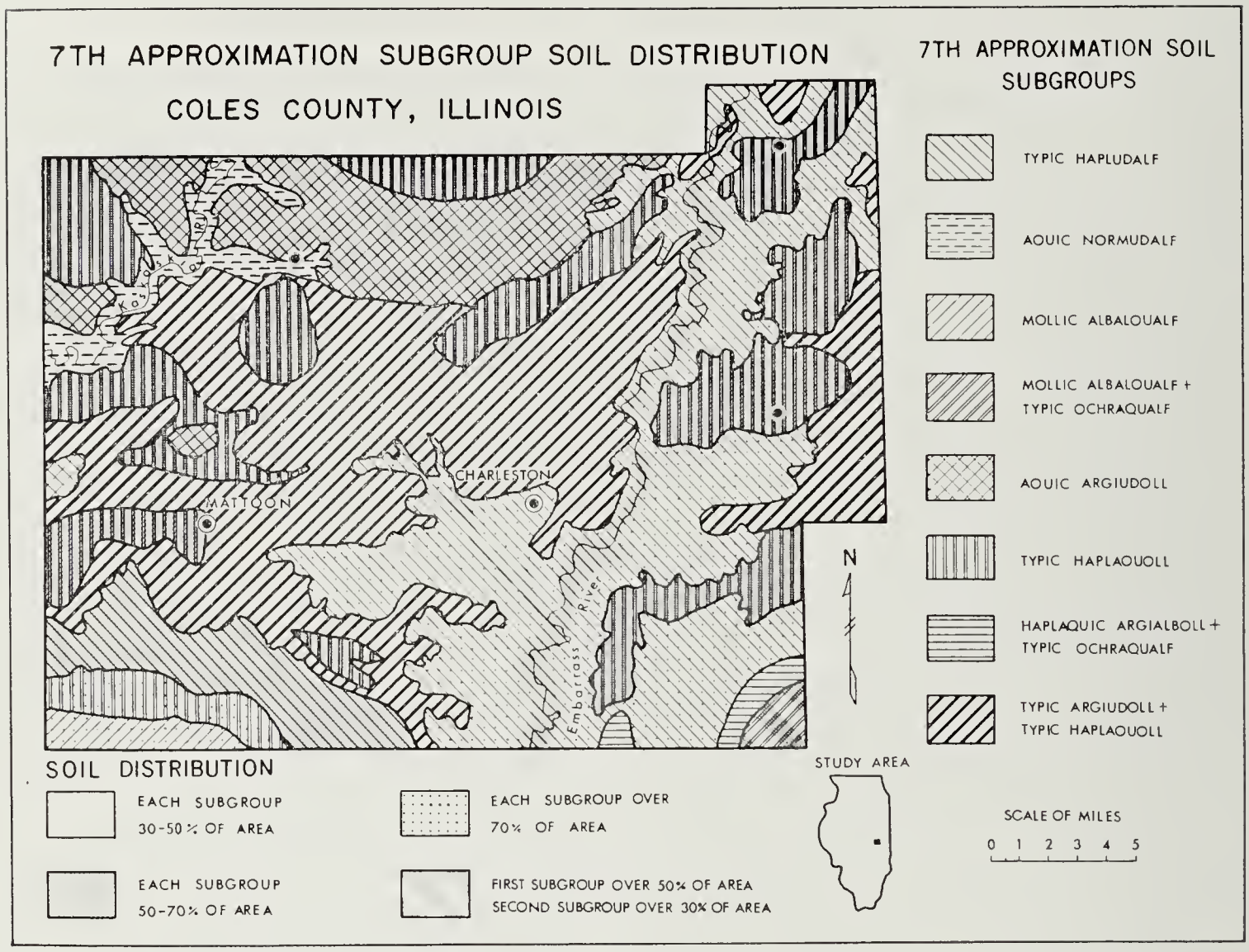

FIGURE 1. — 7th Approximation Subgroup Soil Distribution, Coles County, Illinois.

TABLE 1.—Numerical Values Assigned to the Degree of Soil Limitation in the Construction of Cottages and Utility Buildings.

Degree of Limitation	Numerical Value
Very Severe	5
Severe	4
Moderate	3
Slight	2
Very Slight	1

within a very detailed level of the soil classification framework. Figure 3 shows the extent to which limitation units (Figure 2) deviate (+ or —) from the results obtained from the most accurate expression of limitation which is based on very detailed soil data.

DISCUSSION

In general, the deviations between the detailed soil analysis and the more general subgroup analysis are small. In Figure 2 approximately 15% of the study area has results that are essentially identical (.00-.10 deviation) with the results obtained from a very detailed soil analysis. Another 20% of the study area has a deviation that is so small (.10-.25) that it cannot alter any conclusion in a practical application of a general soil interpretation study. Over 50% of the study area has a more noticeable variation (.25-.50), but again this range of deviation would not alter general land use planning decisions made from Figure 2. In less than 10% of the study area does the deviation exceed .50. The actual maximum calculated .62. Some consideration of error must be taken in these areas but even a .60 deviation

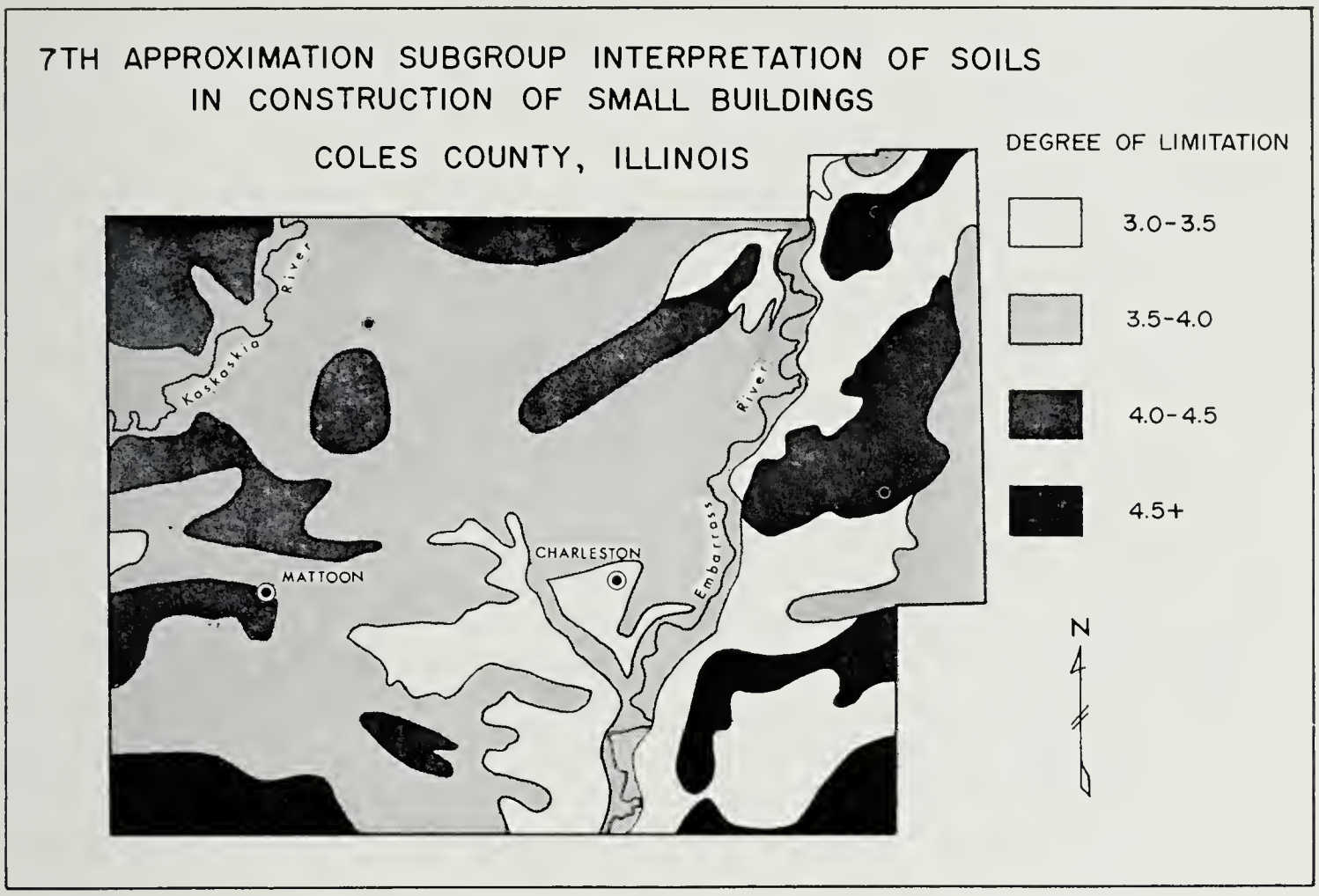

FIGURE 2. — 7th Approximation Subgroup Interpretation of Soils in the Construction of Small Buildings, Coles County, Illinois.

does not change the essential character of limitations required in the relatively "higher" error area. For example, a predicted limitation value of 3.10 northwest of Mattoon is calculated at 3.70, seemingly a large deviation. Yet the error is not excessive when considered within a subjective framework suitable as a broad guide for planning. The other deviations between .00-.50 have little or no change in most subjective definitions. Thus, in the study, a limitation map utilizing 7th Approximation subgroup data is subject to varying degrees of error when compared to a limitation map derived from very detailed soil data. However, the difference between the two maps is small and for general planning purposes the subgroup-based map can be used effectively.

GEOGRAPHICAL INTERPRETATION-SUBGROUP LIMITATION MAP

The study provides a general map for planners and shows in one case that the subgroup level of the 7th Approximation groups soil of such similar character that it can effectively be used in a limitation study. However, the study also provides materials which when analyzed give insight into the interrelationships between the character of the soil and, in this case, its use for constructional purposes. Analysis of the limitation regions and soils which comprise the subgroups within the regions provide the geographer with interpretative information. In Figure 2 the regions with limitations between 3.00 and 3.50 have close association with well-

drained soils, moderate slope and lack of excessively heavy subsoil. Locally severe limitation areas within the region correlate with extreme flatness, excessive slope, excessive erodibility and poor drainage. Soils that were formerly named Gray-Brown Podzolic or well-drained Brunizems areally dominate in the area. Parts of the Shelbyville Moraine and dissected Embarras River Valley area have the best soil character for the construction of cottages and utility buildings.

Limitation areas between 3.50-4.00 are dominated by gently rolling topography, moraine or parts of river valleys. A greater percentage of the soils are ill-drained (as in the swales of the prairie area of flood-plains) compared to the 3.00-3.50 region. Gray-Brown Podzolic soils remain important. The general associations made in region 3.00-3.50 apply in this limitation region also but a noticeable shift to greater roughness or poorer drainage is evident resulting in more severe limitations for the construction of cottages.

Limitation regions 4.00-4.50 have distinctly different associations from the previously discussed regions. Limitations are severe. The soils in this region are ill-drained types with fine-textured soils becoming somewhat dominant. Topographically, areas dominated by swales or other flat features are the most common in this region. Humic Gley soils, Planosol-like soils and some Alluvi-

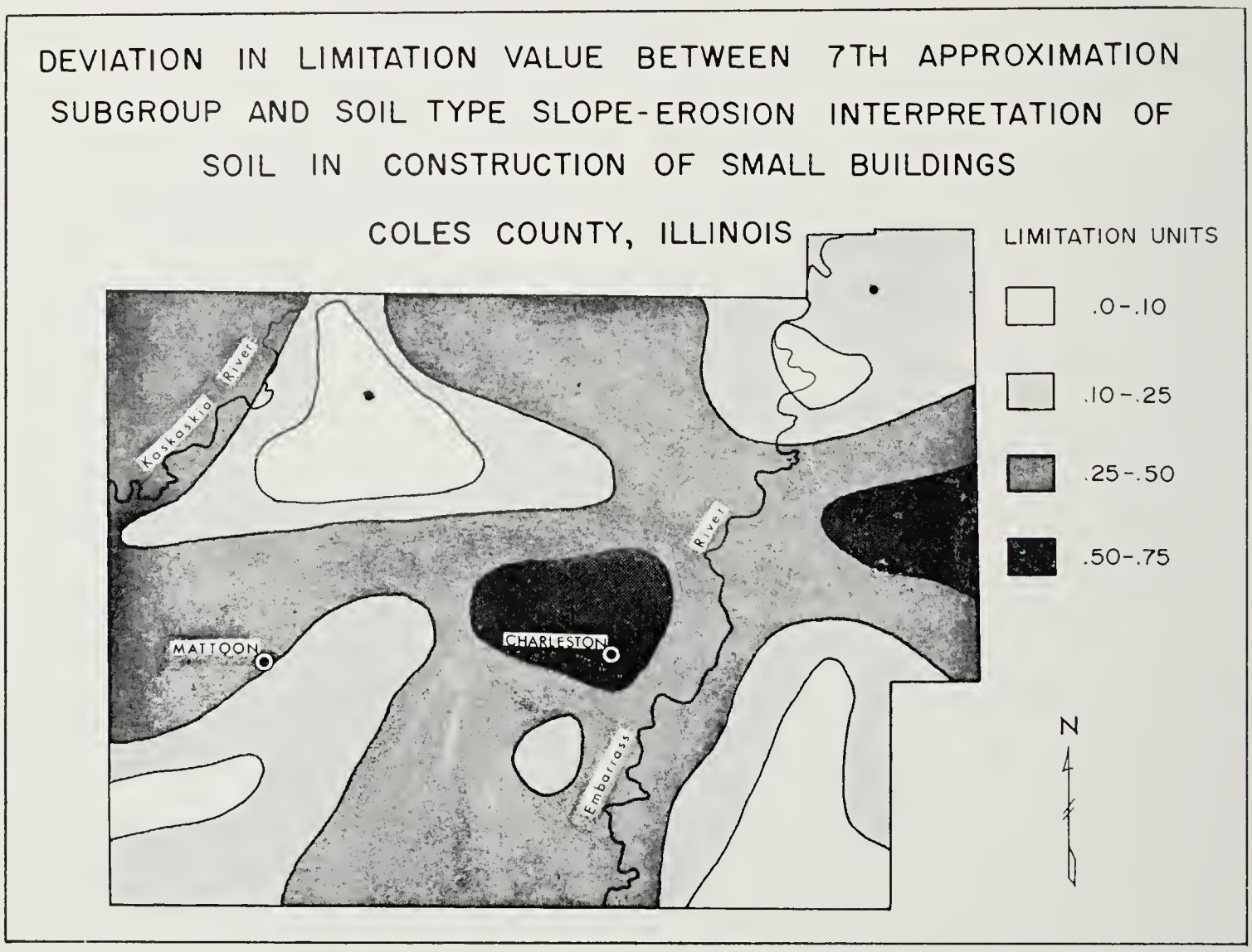

FIGURE 3. — Deviation in Limitation Value Between 7th Approximation Subgroup and Soil Type-Slope-Erosion Interpretation of Soil in the Construction of Small Buildings, Coles County, Illinois.

al soils have the largest areal representation in the region (1949 classification).

Region 4.50-5.00 has very severe limitation for the construction of cottages and utility buildings. In the study area the primary association is between severe limitation and fine-textured soils. Flat terrain and poor drainage are closely associated with the fine-textured soils. This part of the study area is almost completely dominated by Planosol or Planosol-like soils.

From these and other associations it is possible to better understand the factors which are critical in the development of limitation regions. A more detailed analysis of the soil characteristics in the limitation regions can show many more of the intimate interrelationships between soil and the resulting suitability of the land to support a particular land use within its regional framework.

Summary and Conclusions

(1) The results of this study are valid within the given framework which utilizes one county of Illinois, one soil interpretation at one level of the 7th Approximation. Many more detailed studies, using various levels of the 7th Approximation in diverse areas with different types of land use interpretations are required before conclusive statements may be made concerning the applicability of the 7th Approximation in other land limitation studies. This type of application as well as others should be attempted using the 7th Approximation in order to learn more about its character. (2) This study demonstrates conclusively that 7th Approximation subgroup maps can successfully be used for selected land use interpretation studies. (3) Interrelationships between soil characteristics and the suitability of land for constructional purposes can easily and quickly be made by utilizing soils organized within the 7th Approximation subgroup framework. (4) Theoretically, the interpretation of interrelationships between any level of the 7th Approximation and many other earth phenomena is possible in a variety of geographic analyses. Completion of this type of study will aid in a greater geographic understanding of soil and soil-related phenomena.

Acknowledgments

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THE HYDROLYSIS OF ADENOSINETRIPHOSPHATE BY BLUEGILL LIVER MITOCHONDRIA IN THE PRESENCE OF 2,4-DICHLOROPHENOXYACETIC ACID DERIVATIVES

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ABSTRACT. — The effects of the sodium salt, the dimethylamine salt, the butyl ester, the butoxy-ethanol ester, the ethyl ester, the isopropyl ester, the isooctyl ester, the propyleneglycolbutyl-ether ester, and the 2-ethyl-hexyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D) on the hydrolysis of adenosinetriphosphate (ATP) by the bluegill (*Lepomis macrochirus*) liver mitochondria in the presence of five metals were investigated. The butyl and isopropyl esters increased the hydrolysis of ATP in the presence of manganese, magnesium, and calcium; however, the isopropyl ester was more effective than the butyl ester in the presence of magnesium and calcium. The butyl ester did not appreciably alter the hydrolysis of ATP in the presence of cadmium and zinc, but the isopropyl ester inhibited the hydrolysis of ATP in the presence of both metals. The dimethylamine salt was the least effective of all the derivatives investigated, and the other derivatives varied in their effect on the hydrolysis of ATP in the presence of the various metals.

The hydrolysis of adenosinetriphosphate (ATP) by bluegill (*Lepomis macrochirus*) liver mitochondria was recently reported (Hiltibran 1966) and was part of an investigation to determine the effect of pollutants on fishes.

The data of Hughes and Davis (1963) and Lawrence (1962) indicated that some derivatives of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) were more toxic to bluegills than were other 2,4-D derivatives. Similar data had been obtained in our laboratory and recently was reported (Hiltibran 1967*a*).

Since some of the 2,4-D derivatives had been shown to be more toxic to small bluegills than other 2,4-D derivatives, it would appear that the more toxic 2,4-D derivatives might alter some biochemical activity and these changes could be estimated. An investigation of the effects of nine 2,4-D derivatives on the hydrolysis of ATP by the mitochondria from bluegill liver in the presence of cadmium, zinc, manganese, magnesium and calcium was initiated. In addition, the effects of the nine derivatives of 2,4-D on succinic acid oxidase and the alpha-Ketoglutaric acid oxidase enzyme complexes from the mitochondria of bluegill liver were investigated and are summarized elsewhere (Hiltibran 1968).

MATERIALS AND METHODS

Native wild bluegills were held in the laboratory in aerated aquaria at 25° C and all enzymatic assays were conducted

at the same temperature. Procedures for the preparation of the mitochondria, for estimating the rate of release of inorganic phosphate from ATP, and for estimating the nitrogen content of the mitochondrial preparations have been reported (Hiltibran and Johnson 1965). The amounts of inorganic phosphate released from the ATP were converted to micromoles of ATP hydrolyzed per hour per milligram of tissue nitrogen. The data are the average change observed from three or more experiments and have been corrected for endogenous activity and the effects of the solvent. The technical grade or pure derivatives were obtained from various manufacturers and were used without further purification. Redistilled ethyl alcohol and acetone were used as solvents.

Results and Discussion

The effects of various 2,4-D derivatives on the cadmium-enhanced hydrolysis of ATP are summarized in

Table 1.—The Effects of 2, 4-D Derivatives on the Hydrolysis of ATP in the Presence of Cadmium

2, 4-D Derivative	μmoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in μmoles ATP/hr/mgN		
Na salt	—38	—37	—48
# exp.	(4)	(5)	(6)
Range	((—) 8 — (—) 90)	((—) 12 — (—) 76)	((—) 3 — (—) 104)
Dimethylamine "salt"	±7	±19	±22
# exp.	(3)	(3)	(3)
Range	((—) 2 — (+) 15)	((—) 6 — (+) 37)	((+) 24 — (—) 40)
Butyl ester	±6	±11	±3
# exp.	(4)	(3)	(3)
Range	((+) 8 — (—) 14)	((—) 13 — (+) 15)	((—) 2 — (+) 6)
Butoxy—ethanol ester	—11	—19	—13
# exp.	(3)	(3)	(5)
Range	((—) 10 — (—) 14)	((—) 8 — (—) 39)	(0 — (—) 39)
Ethyl ester	.....	—41	—65
# exp.	.....	(3)	(3)
Range	.....	((—) 13 — (—) 65)	((—) 35 — (—) 100)
Isopropyl ester	—15	—27	—45
# exp.	(4)	(7)	(3)
Range	((—) 6 — (—) 37)	((—) 6 — (—) 54)	((—) 22 — (—) 71)
Isooctyl ester	±11	±30	±26
# exp.	(4)	(6)	(4)
Range	((+) 7 — (—) 13)	((+) 21 — (—) 42)	((+) 20 — (—) 43)
PGBE ester	—14	—20	—29
# exp.	(4)	(4)	(3)
Range	((—) 5 — (—) 33)	((—) 9 — (—) 31)	((—) 15 — (—) 51)
2-ethyl-hexyl ester	±3	—23	—28
# exp.	(4)	(4)	(3)
Range	((—) 13 — (+) 5)	((—) 13 — (—) 28)	((—) 22 — (—) 35)

TABLE 2.—The Effects of 2, 4-D Derivatives on the Hydrolysis of ATP in the Presence of Zinc.

2, 4-D Derivatives	μmoles of Reaction Medium 1.5	2.5	5.0
	Average Change in μmoles/ml/ATP/hr/mgN		
Na salt	—25	—34	—35
# exp.	(3)	(4)	(4)
Range	((—) 24 — (—) 28)	((—) 27 — (—) 39)	((—) 2 — (—) 64)
Dimethylamine salt	±10	±13	±21
# exp.	(3)	(5)	(5)
Range	((+) 9 — (—) 20)	((+) 5 — (—) 28)	((+) 2 — (—) 47)
Butyl ester	±13	±7	±15
# exp.	(7)	(6)	(6)
Range	((+) 0.6 — (—) 28)	((—) 2 — (+) 17)	((—) 22 — (+) 21)
Butoxy-ethanol ester	—17	—14	—17
# exp.	(3)	(4)	(4)
Range	((—) 8 — (—) 31)	((—) 7 — (—) 18)	((—) 4 — (—) 25)
Ethyl ester		—22	—46
# exp.		(3)	(3)
Range		((—) 5 — (—) 35)	((—) 38 — (—) 54)
Isopropyl ester	—10	—21	—7
# exp.	(5)	(5)	(4)
Range	((—) 0 — (—) 23)	((—) 6 — (—) 36)	((—) 4 — (—) 11)
Isooctyl ester	—10	—15	—21
# exp.	(5)	(6)	(5)
Range	(0.3 — (—) 22)	((—) 2 — (—) 59)	((—) 8 — (—) 53)
PGBE ester	±5	±18	±18
# exp.	(5)	(8)	(5)
Range	((—) 6 — (+) 8)	((—) 33 — (+) 27)	((—) 45 — (+) 13)
2-ethyl-hexyl ester	±7	±10	±3
# exp.	(5)	(5)	(4)
Range	((—) 3 — (+) 20)	((—) 5 — (+) 15)	((+) 0.2 — (—) 6)

Table 1. The dimethylamine salt and the isooctyl ester did not alter the hydrolysis of ATP. Six derivatives inhibited the hydrolysis of ATP, and the butyl ester only at high levels inhibited the hydrolysis of ATP.

The effects of the 2,4-D derivatives on the hydrolysis of ATP in the presence of zinc are summarized in Table 2. The butyl ester, PGBE ester and the 2-ethyl-hexyl ester did not alter the hydrolysis of ATP. The dimethylamine salt at high levels increased the hydrolysis of ATP. The other derivatives at all levels investigated inhibited the hydrolysis of ATP.

The effects of 2,4-D derivatives on the hydrolysis of ATP in the presence of manganese are summarized in Table 3. The sodium salt and the butoxy-ethanol ester inhibited the hydrolysis of ATP. The dimethylamine

salt, the ethyl ester, and the isooctyl ester did not alter the hydrolysis of ATP. The butyl ester, the isopropyl ester, the PGBE ester and the 2-ethyl-hexyl ester at high levels increased the hydrolysis of ATP.

The effects of the 2,4-D derivatives on the hydrolysis of ATP in the presence of magnesium are summarized in Table 4. The sodium salt, the dimethylamine salt, the butoxy-ethanol ester and the ethyl ester did not alter the hydrolysis of ATP. The butyl ester, isopropyl ester and 2-ethyl-hexyl ester at all levels utilized increased the hydrolysis of ATP, whereas the isooctyl ester and the PGBE ester at high levels increased the hydrolysis of ATP.

The effects of the 2,4-D derivatives on the hydrolysis of ATP in the presence of calcium are summarized in

TABLE 3.—The Effects of 2, 4-D Derivatives on the Hydrolysis of ATP in the Presence of Manganese.

2, 4-D Derivative	μmoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in μmoles ATP/hr/mgN		
Na salt	—27	—27	—38
# exp.	(3)	(3)	(3)
Range	((—) 17 — (—) 37)	((—) 14 — (—) 37)	((—) 22 — (—) 54)
Dimethylamine salt	±12	±7	±6
# exp.	(3)	(3)	(3)
Range	((—) 6 — (+) 22)	((—) 2 — (+) 11)	((—) 1 — (+) 8)
Butyl ester	±3	+23	+45
# exp.	(4)	(4)	(4)
Range	((—) 5 — (+) 6)	((+) 8 — (+) 33)	((+) 12 — (+) 93)
Butoxy-ethanol ester	—13	—22	—33
# exp.	(3)	(7)	(3)
Range	((—) 4 — (—) 28)	((—) 8 — (—) 30)	((—) 20 — (—) 46)
Ethyl ester	.....	±21	±17
# exp.	.....	(5)	(3)
Range	.....	((—) 20 — (+) 45)	((+) 2 — (—) 42)
Isopropyl ester	±18	+35	+47
# exp.	(4)	(6)	(4)
Range	((—) 4 — (+) 59)	((+) 2 — (+) 105)	((+) 36 — (+) 68)
Isooctyl ester	±7	±16	±18
# exp.	(4)	(7)	(4)
Range	((—) 2 — (+) 10)	((+) 1 — (—) 23)	((—) 3 — (+) 50)
PGBE ester	±10	±18	+21
# exp.	(4)	(7)	(4)
Range	((—) 13 — (+) 13)	((—) 30 — (+) 26)	((+) 14 — (+) 27)
2-ethyl-hexyl ester	±8	±8	+40
# exp.	(4)	(5)	(3)
Range	((—) 2 — (+) 16)	((—) 3 — (+) 2)	((+) 10 — (+) 63)

TABLE 4.—The Effects of 2, 4-D Derivatives on the Hydrolysis of ATP in the Presence of Magnesium.

2, 4-D Derivative	μmoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in μmoles ATP/hr/mgN		
Na salt	±5	±8	±7
# exp.	(4)	(6)	(4)
Range	((—) 16 — (+) 12)	((—) 17 — (+) 7)	((+) 5 — (—) 10)
Dimethylamine salt	±17	±15	±19
# exp.	(3)	(4)	(4)
Range	((+) 3 — (—) 42)	((+) 4 — (—) 50)	((+) 3 — (—) 56)
Butyl ester	+33	+41	+48
# exp.	(4)	(5)	(4)
Range	((+) 15 — (+) 54)	((+) 23 — (+) 67)	((+) 29 — (+) 82)
Butoxy-ethanol ester	±12	±20	±13
# exp.	(3)	(5)	(4)
Range	((—) 2 — (+) 18)	((—) 3 — (+) 61)	((—) 3 — (+) 28)
Ethyl ester		±3	±6
# exp.		(4)	(4)
Range		((—) 1 — (+) 10)	((—) 4 — (+) 16)
Isopropyl ester	+49	+51	+28
# exp.	(4)	(4)	(4)
Range	((+) 31 — (+) 74)	((+) 36 — (+) 82)	((+) 5 — (+) 45)
Isooctyl ester	±4	±2	+19
# exp.	(5)	(4)	(4)
Range	((—) 8 — (+) 2)	((—) 2 — (+) 3)	((+) 6 — (+) 29)
PGBE ester	±10	±10	+36
# exp.	(4)	(6)	(4)
Range	((—) 4 — (+) 17)	((—) 8 — (+) 20)	((+) 6 — (+) 67)
2-ethyl-hexyl ester	+25	+19	+50
# exp.	(4)	(3)	(3)
Range	((+) 2 — (+) 54)	((+) 2 — (+) 66)	((+) 34 — (+) 66)

Table 5. The sodium salt, the dimethylamine salt and the isooctyl ester did not alter the hydrolysis of ATP. The isopropyl ester at all levels investigated increased the hydrolysis, whereas the other esters only at high levels increased the hydrolysis of ATP.

Previously reported micromoles of ATP hydrolyzed per hour per milligrams of tissue nitrogen are as follows: cadmium 90, zinc 70, manganese 50, magnesium 28, calcium 24 (Hiltibran, 1966). Therefore, the maximum inhibition of the hydrolysis of ATP by the ethyl ester in the presence of cadmium and zinc was approximately 70 percent. Butoxy-ethanol ester inhibited the hydrolysis of ATP in the presence of manganese approximately 60 percent. The maximum increase in the hydrolysis of

ATP in the presence of magnesium by low levels of the butyl ester and the isopropyl ester was approximately 120 and 150 percent respectively. The maximum increase of the hydrolysis of ATP by the butyl ester in the presence of calcium was about 100 percent.

Since both the butyl ester and the isopropyl ester at low levels (1.5 μmoles) altered the hydrolysis of ATP in the presence of magnesium, the effect of 0.15 and 0.25 μmoles of these esters on the hydrolysis of ATP were investigated. The isopropyl ester at a level of 0.15 μmoles increased the hydrolysis approximately 30 percent, whereas the butyl ester was not effective. Both esters increased the release of inorganic phosphate from ATP at the 0.25 μmoles level and above.

TABLE 5.—The Effects of 2, 4-D Derivatives on the Hydrolysis of ATP in the Presence of Calcium.

2, 4-D Derivatives	μmoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in μmoles ATP/hr/mgN		
Na salt	±8	±4	±5
# exp.	(3)	(5)	(5)
Range	((—) 3 — (+) 12)	((—) 12 — (+) 2)	((—) 16 — (+) 2)
Dimethylamine salt	±6	±8	±3
# exp.	(3)	(3)	(4)
Range	((—) 1 — (+) 14)	((—) 6 — (+) 14)	((+) 4 — (—) 9)
Butyl ester	±4	±15	+25
# exp.	(6)	(5)	(4)
Range	((—) 1 — (+) 10)	((—) 3 — (+) 49)	((+) 3 — (+) 50)
Butoxy-ethanol ester	±5	±6	+10
# exp.	(3)	(5)	(3)
Range	((—) 0.2 — (+) 13)	((—) 0.7 — (+) 26)	((+) 8 — (+) 12)
Ethyl ester		±5	+10
# exp.		(3)	(3)
Range		((—) 2 — (+) 10)	((+) 7 — (+) 11)
Isopropyl ester	+13	+16	+17
# exp.	(4)	(4)	(3)
Range	((+) 2 — (+) 31)	((+) 4 — (+) 45)	((+) 11 — (+) 29)
Isooctyl ester	±2	±6	±2
# exp.	(4)	(5)	(4)
Range	((—) 5 — (+) 1)	((—) 6 — (+) 8)	((—) 1 — (+) 4)
PGBE ester	±3	±7	+14
# exp.	(3)	(6)	(3)
Range	((+) 2 — (—) 6)	((—) 8 — (+) 13)	((+) 10 — (+) 17)
2-ethyl-hexyl ester	±3	±4	+14
# exp.	(4)	(4)	(3)
Range	((—) 1 — (+) 6)	((—) 7 — (+) 4)	((+) 8 — (+) 18)

The data also indicated that various derivatives of 2,4-D can alter the hydrolysis of ATP in the presence of various metals to different extents and in different ways. The data also indicates that high levels of many of the derivatives used in this investigation can alter the hydrolysis of ATP.

The biochemical importance of the hydrolysis of ATP in the presence of cadmium, zinc, manganese and calcium is not known. Magnesium appears to be involved in the conversion of energy produced by the oxidation of substrates into useful energy, presumably via ATP or other phosphate intermediates. Therefore, the effects of the various 2,4-D derivatives on the hydrolysis of ATP in the presence of magnesium may be important.

The effect of cadmium and zinc on the hydrolysis of ATP cannot be discounted. In the previous studies (Hiltibran 1965, 1967b) cadmium and zinc at very low levels inhibited the oxygen uptake in the presence of succinate and alpha-ketoglutarate and caused large increases in the phosphate content of the reaction vessels, i.e. greater than the phosphate contents observed when oxygen uptake was inhibited by sodium cyanide or other inhibitors which did not alter the hydrolysis of phosphate intermediates. These data indicated that cadmium and zinc may have had a two-fold action, i.e. one on the inhibition of oxygen uptake, and a second one on the hydrolysis of phosphate intermediates. The latter was confirmed when it was found that cadmium and zinc increased the hydrolysis of ATP (Hiltibran 1966). It would appear, therefore, that the greater toxicity of cadmium to bluegills may be due to the greater disruption of energy production by cadmium than the disruption of energy production by zinc. Similar observations are available for manganese and calcium although the effects of these metals on the oxygen uptake and hydrolysis of phosphate intermediates were less severe. It would appear that the isopropyl ester could disrupt energy production to a greater extent than the other 2,4-D derivatives. This would appear to correlate with the greater toxicity of the isopropyl ester to bluegills than the toxicity of the other 2,4-D derivatives to bluegills (Hughes and Davis 1963).

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THE PEACOCK PRAIRIE — A STUDY OF A VIRGIN ILLINOIS MESIC BLACK-SOIL PRAIRIE FORTY YEARS AFTER INITIAL STUDY

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ABSTRACT. — The Peacock Prairie is a 5½ acre tract of land in Cook County, 4 acres of which are comparatively high quality mesic black soil prairie. According to historical records and to testimony of the descendants of the Peacock family, the original owners of the property, the land has never been plowed or systematically grazed. The nature of the vegetative cover tends to support this view. Although of comparatively small size, this tract of land is probably the largest virgin *mesic black-soil* prairie extant in Illinois. The few weeds that were found on the prairie in 1926-27, resulting from light grazing, had practically disappeared by 1967. Peacock Prairie contains approximately 130 species of comparatively rare "prairie plants," including ten species of prairie legumes, two lilies, two gentians, and one orchid. In the forty-year period (1927-1967) only one species of prairie plant seemed to have disappeared; however, nineteen species, not reported in 1927, were found on the prairie in 1967. It is remarkable that with no care whatsoever (e.g. no controlled burning or mowing) the prairie has resisted the invasion of both weeds and woody plants; consequently, it offers a norm or standard of comparison for future studies of prairie and prairie restoration.

There have been surprisingly few studies made of Illinois prairies. Gleason (1910) studied the sand prairies of the inland sand deposits, and Gates (1912) the sand prairies of the beach area in northeastern Illinois. Evers (1955) studied the hill prairies along the Illinois River and Fell & Fell (1956) examined those along the Rock River; yet with the exception of the studies of Vestal (1914) and Sampson (1921), there have been no studies of the virgin Illinois black-soil prairies, which at one time represented the major portion of the vegetation of northern and central Illinois. The survival of a virgin black-soil prairie in Illinois is extremely fortuitous because most of them have been practically destroyed through overgrazing and cultivation during the past 150 years of settlement.

In 1929, Paintin published a paper about a small prairie in northeastern Illinois which occupied a tract of land that was part of the Peacock estate and which was (and still is) known as the Peacock Prairie. It is located on the southwest outskirts of Glenview in Cook County and is a virgin black-soil prairie which contains patches of low prairie. At the time of the initial study (1926-27) the prairie was approximately ten acres, but during the intervening years the eastern part has been destroyed for a housing development;

today the prairie is about 5½ acres, 4 of which are high quality virgin prairie. In Paintin's paper the prairie was described, the plants found on it were recorded, and an effort was made to quantify the collected data.

Forty years have elapsed since the original study was made, and it would seem timely to make a comparative study to determine what changes, if any, have occurred during this period.

Topography and Composition of the Prairie

Mr. Warren Long, a direct descendant (fifth generation) of the original owner, has stated that as far as he knows, the land (Peacock Prairie) has never been broken, fenced, or grazed. He further notes that it was traversed by a wagon exit from the farm, which lay to the east, onto Milwaukee Avenue (Indian Trail Road) at one time. The prairie is located on Tinley ground moraine. The soil is developed on calcareous silty clay till and borings made at the site show the leached, or "A" zone, to extend to a depth of approximately 20 inches. The upper 15 inches is black, organic rich, soil.

The prairie's original topographic surface is still evident with a low swale containing typically low prairie vegetation in the western section and with moderately higher knolls covered with upland or mesic prairie vegetation elsewhere. Upland prairie grasses, such as *Stipa spartea, Sorghastrum nutans, Andropogon gerardii, Andropogon scoparius* and *Panicum virgatum*, and those of the lowland, such as *Calamagrostis canadensis* and *Spartina pectinata*, produce a short thick sward about 2-3 feet high. Many of these grasses produce relatively few flowering culms in this dense prairie sward. Similarly, many of the late blooming composites, such as *Liatris* sp. flower sparsely under these conditions.

The 129 species of native prairie plants and 21 species of weeds that have been found and recorded for the Peacock Prairie and listed in Table 1 together with a comparison of their relative frequencies in 1927 and 1967. Among the 129 species of native prairie plants, 19 of them were not reported by Paintin in 1927. They are:

- Andropogon scoparius (little bluestem)
- Asclepias incarnata (swamp milkweed)
- Asclepias tuberosa (butterfly weed)
- Desmodium illinoense (Illinois tick trefoil)
- Galium boreale (Northern bedstraw)
- Gentiana andrewsii (closed gentian)
- Gerardia tenuifolia (purple false foxglove)
- Hypericum canadense (Canadian St. John's wort)
- Juncus acuminatus (sharp-fruited rush)
- Juncus tenuis (roadside rush)
- Lathyrus palustris (marsh vetchling)
- Lespedeza capitata (round-headed bush clover)

Table 1. — Changes in the Vascular Plants of the Peacock Prairie during the Period 1927-1967.

Since the terms "abundant", "frequent", "infrequent", and "local" were used in describing the relative frequencies of the plants found on the prairie in 1927, the same terms and, in addition, the term "rare" are used in this paper to describe the relative frequencies of the plants. Paintin did not give meanings of these terms in her paper, hence, it is not known whether they have the same approximate meanings as are intended by the present authors. In this paper the terms are used to indicate the relative frequencies of each species in the Peacock Prairie based on their individual patterns of occurrence as observed in the midwestern prairies studies by the authors. For example, in an acre of prairie, 25 plants of big bluestem grass (*Andropogon gerardii*) would be considered "infrequent" while to find 25 plants of the prairie lily (*Lilium philadelphicum* var. *andinum*) in an acre would be "common". The same number of Mead's milkweed (*Asclepias meadii*) would certainly be "abundant" for that species. Since each species is characterized by unique habits of growth, there is no valid way of comparing them all on a single absolute numerical scale.

Nomenclature for this paper is in accordance with Gray's Manual of Botany, 8th Edition, except for occasional alteration of single "i" endings to double "i" endings as recommended by the current rules of nomenclature.

Table 1.

Prairie Plants	1927	1967
Agrostis alba (w)	frequent	frequent, local
Agrostis hyemalis (w)	abundant	infrequent, local
Allium canadense (w)	abundant	infrequent
Allium cernuum	frequent	frequent
Amorpha canescens	infrequent	frequent
Andropogon gerardii	frequent	frequent
Andropogon scoparius (d)	.....	frequent
Anemone cylindrica	infrequent	infrequent
Antennaria neglecta	abundant	infrequent, local
Apocynum cannabinum	frequent	frequent
Asclepias incarnata (w)	.....	frequent, local
Asclepias sullivantii (w)	frequent	frequent
Asclepias tuberosa	.....	infrequent
Aster azureus	infrequent	rare
Aster ericoides	frequent	frequent
Aster laevis	frequent	abundant
Aster novae-angliae	infrequent	infrequent
Aster ptarmicoides (d)	infrequent	infrequent
Aster simplex (w)	abundant, local	infrequent, local
Baptisia leucantha	infrequent	infrequent
Baptisia leucophaea	frequent	frequent
Bidens frondosa (w)	frequent	frequent, local
Bromus kalmii	infrequent	frequent
Cacalia tuberosa	infrequent	infrequent
Carex buxbaumii (w)	infrequent, local	infrequent, local
Carex crawei (w)	infrequent, local	infrequent, local
Carex vulpinoidea (w)	infrequent, local	infrequent, local
Calamagrostis canadensis (w)	frequent	abundant, local
Cicuta maculata (w)	infrequent	infrequent
Cirsium hillii	frequent	infrequent
Comandra richardsiana	abundant	abundant
Coreopsis palmata	abundant	abundant
Desmodium illinoense (d)	.....	rare
Dodecatheon meadia	infrequent	frequent
Eleocharis palustris (w)	abundant	infrequent, local
Elymus canadensis	infrequent	infrequent
Equisetum arvense	frequent	rare, local
Eryngium yuccifolium	frequent	frequent
Fragaria virginiana	frequent	frequent
Galium obtusum (w)	infrequent	frequent
Galium boreale	.....	infrequent
Gentiana andrewsii (w)	.....	frequent
Gentiana puberula	frequent	frequent
Gerardia aspera	rare, 2 plants	.....
Gerardia tenuifolia	.....	infrequent, local

w—wet
d—dry

TABLE 1.—Continued.

Prairie Plants	1927	1967
Geum triflorum (d)	rare	infrequent
Glyceria striata (w)	frequent	infrequent
Helianthus grosseserratus	abundant	infrequent
Helianthus laetiflorus v. rigidus	abundant	frequent
Heuchera richardsonii	frequent	frequent
Hypericum canadense (w)	.....	rare, 1 plant
Hypoxis hirsuta	abundant	frequent
Juncus acuminatus (w)	.....	infrequent, local
Juncus balticus v. littoralis (w)	abundant	infrequent, local
Juncus dudleyi (w)	frequent	infrequent, local
Juncus tenuis	.....	infrequent, local
Juncus torreyi (w)	infrequent	infrequent, local
Koeleria cristata (d)	infrequent	frequent
Krigia biflora (w)	frequent	frequent
Kuhnia eupatorioides (d)	infrequent	rare
Lathyrus palustris	.....	infrequent
Lactuca canadensis	frequent	rare
Lespedeza capitata (d)	.....	infrequent
Liatris aspera	frequent	frequent
Liatris spicata	infrequent	frequent
Lilium michiganense (w)	infrequent	infrequent
Lilium philadelphicum v. andinum	infrequent	infrequent
Lithospermum canescens	frequent	frequent
Lobelia spicata	frequent	frequent
Ludwigia polycarpa (w)	abundant, local	infrequent, local
Lycopus americana (w)	frequent	frequent, local
Lysimachia ciliata (w)	frequent, local	infrequent, local
Lysimachia quadriflora (w)	abundant	infrequent
Lythrum alatum (w)	abundant	frequent
Monarda fistulosa	.....	frequent
Oxalis stricta	rare	rare
Oxalis violacea	frequent	frequent
Oxypolis rigidior (w)	infrequent	infrequent
Panicum lanuginosum (w)	infrequent	infrequent, local
Panicum leibergii	infrequent	frequent
Panicum virgatum	frequent	frequent
Parthenium integrifolium	infrequent	frequent
Pedicularis canadensis	frequent	frequent
Penthorum sedoides (w)	frequent, local	rare, local
Petalostemum candidum	frequent	abundant
Petalostemum purpureum	frequent	abundant
Phlox glaberrima (w)	abundant	frequent
Phlox pilosa	frequent	frequent
Polygala senega	frequent	frequent, local
Polygonum amphibium v. stipulaceum (w)	abundant, local	frequent, local

w—wet
d—dry

TABLE 1. — Continued.

Prairie Plants	1927	1967
Potentilla arguta	infrequent	infrequent
Potentilla simplex	abundant	infrequent, local
Prenanthes aspera	frequent	infrequent
Prenanthes racemosa (w)	infrequent	infrequent
Prunella vulgaris v. lanceolata (w)	infrequent	infrequent, local
Pycnanthemum virginianum (w)	abundant	frequent
Rosa carolina	frequent	frequent
Ratibida pinnata	frequent	infrequent
Rudbeckia hirta	abundant	infrequent
Scirpus lineatus (w)	.....	infrequent, local
Scutellaria parvula v. leonardii	.....	infrequent
Senecio pauperculus v. balsamitae	abundant	frequent, local
Sphenopholis obtusata (w)	.....	infrequent
Silphium integrifolium	abundant	frequent
Silphium laciniatum	frequent	frequent
Silphium terebinthinaceum	frequent	frequent
Sisyrinchium albidum	abundant	frequent
Smilacina stellata	abundant	frequent
Solidago altissima (canadensis)	infrequent	infrequent, local
Solidago graminifolia (w)	infrequent	infrequent
Solidago nemoralis	frequent	frequent
Solidago riddellii (w)	abundant	frequent
Solidago rigida	frequent	frequent
Sorghastrum nutans	frequent	abundant
Spartina pectinata (w)	abundant	frequent, local
Spiranthes cernua	rare, 2 plants	rare, 1 plant
Sporobolus heterolepis	.....	frequent
Stachys palustris (w)	abundant, local	infrequent, local
Stipa spartea	frequent	abundant
Thalictrum dasycarpum (w)	.....	rare
Tradescantia ohioensis	infrequent	infrequent
Typha latifolia (w)	infrequent	infrequent, local
Valeriana ciliata (w)	.....	rare
Verbena hastata (w)	infrequent	rare
Veronicastrum virginicum	abundant	infrequent
Vicia americana	frequent	infrequent
Viola papilionacea	abundant	frequent
Viola pedatifida	abundant	frequent
Zizia aurea (w)	frequent	frequent

Monarda fistulosa (wild bergamot)
Scirpus lineatus (red bulrush)
Scutellaria parvula v. leonardii (small skullcap)
Sphenopholis obtusata (wedge grass)
Sporobolus heterolepis (Northern dropseed)
Thalictrum dasycarpum (meadow rue)
Valeriana ciliata (valerian)

It is remarkable that the very characteristic and conspicuous prairie grasses, *Andropogon scoparius* and *Sporobolus heterolepis*, were not observed by Paintin in 1927.

TABLE 1.—Concluded.

Prairie Plants	1927	1967
Eurasian Weeds		
Achillea millefolium	frequent	infrequent, local
Asparagus officinalis	infrequent, edge	.....
Chrysanthemum leucanthemum v. pinnatifidum	abundant, edge	.....
Cirsium arvense	infrequent, edge	.....
Festuca elatior	rare	.....
Medicago sativa	rare, local	.....
Melilotus alba	infrequent	rare
Phleum pratense	infrequent, local	.....
Poa compressa	infrequent	rare, local
Poa pratensis	infrequent	infrequent, local
Rumex crispus	rare, edge	.....
Taraxacum officinale	infrequent	.....
Tragopogon pratensis	rare	.....
Trifolium hybridum	frequent	.....
Trifolium pratense	frequent	.....
Native Weeds		
Ambrosia artemisiifolia	infrequent	rare, local
Erigeron strigosus	infrequent, edge	rare
Hordeum jubatum (west)	infrequent, edge	.....
Oenothera biennis	infrequent	.....
Plantago rugelii	rare	.....
Xanthium pennsylvanicum	infrequent	.....

Seventeen species of plants which were reported by Paintin but not found in 1967 are listed below. Several of these species are far from their natural range in the Chicago area. The authors have indicated in parentheses species which may have been misidentified by Paintin.

Aster commutatus (Aster ericoides?)
Aster oblongifolius
Galium tinctorium (Galium obtusum?)
Gerardia aspera
Gentiana affinis (Gentiana puberula?)
Lactuca campestris (Lactuca canadensis?)
Lactuca hirsuta (Lactuca canadensis?)
Liatris graminifolia (Liatris spicata?)
Oxalis cymosa (Oxalis stricta?)
Petalostemum tenuifolium (Petalostemum purpureum?)
Pycnanthemum pilosum (Pycnanthemum virginianum?)
Rosa humilis (Rosa carolina)
Rosa woodsii (Rosa carolina)
Senecio plattensis (Senecio pauperculus?)
Sisyrinchium campestre (Sisyrinchium albidum?)
Viola cucullata (Viola papilionacea?)
Xanthium commune (Xanthium pensylvanicum?)

The following three species are identified by Paintin to genus, but not to species:

Aster sp. (white) (Aster simplex?)
Equisetum sp. (Equisetum arvense?)
Viola sp.

Many of the species of low prairie, such as, *Ludwigia polycarpa* and *Penthorum sedoides*, are less common today than in 1927, undoubtedly due to a lowering of the water table brought about by changes in drainage. Mr. Long stated that in former days (1910) the prairie was much wetter than it is today.

Most of the weeds recorded in 1927

seem to have disappeared from the prairie proper by 1967. Only 6 of the 21 species of weeds reported in 1927 were still to be found on the prairie. They are either rare or infrequent. It is possible that they may owe their continued presence on the prairie today to slight disturbances caused by man.

In an effort to give some quantitative estimation of the rich vegetative cover, 5 one-meter quadrats at random locations were staked and the non-graminecus vascular plants counted. In addition, the total number of grass shoots (tillers) were counted in a one-decimeter quadrat. These quadrat studies are listed in Table II.

With the exception of a low swale covered with *Calamagrostis canadensis* (blue joint grass), most quadrats contained from 18 to 24 different species of plants. In addition, most of the quadrats exhibited dense growth of the prairie grasses, including the rare *Panicum leibergii*, which is probably more indicative of a high-quality black-soil prairie than is *Andropogon gerardii* (big bluestem).

TABLE 2.—Counts of Vascular Plants in Various Quadrats on the Peacock Prairie.

Quadrat #1
(1 meter square)—N. W. Cent.

5 Amorpha canescens
10 Aster ericoides
14 Aster laevis
43 Coreopsis palmata
1 Cornus racemosa (seedling)
1 Lithospermum canescens
20 Panicum leibergii
2 Panicum virgatum
4 Parthenium integrifolium
1 Petalostemum candidum
8 Petalostemum purpureum
2 Ratibida pinnata
1 Rosa carolina
1 Silphium terebinthinaceum
Spartina pectinata
1 Viola pedatifida

Quadrat #2
(1 meter square)—West Cent.

3 Allium cernuum
1 Apocynum cannabinum
7 Aster laevis
1 Eleocharis palustris
18 Fragaria virginiana
Glyceria striata
1 Lithospermum canescens
2 Liatris aspera
10 Lysimachia quadriflora
38 Panicum leibergii
6 Pedicularis canadensis
2 Phlox glaberrima
1 Polygala senega
5 Ratibida pinnata
7 Silphium terebinthinaceum
6 Smilacina stellata
6 Solidago riddellii
1 Solidago rigida
Spartina pectinata
1 Viola papilionacea

Quadrat #3
(1 meter square)—East. Cent.

3 Allium cernuum
51 Andropogon scoparius
1 Aster ericoides
24 Aster laevis
8 Comandra richardsiana
26 Coreopsis palmata
3 Eryngium yuccifolium
10 Gentiana puberula
5 Koeleria cristata
10 Liatris aspera
4 Lithospermum canescens
3 Lobelia spicata
11 Panicum leibergii
3 Parthenium integrifolium
17 Petalostemum purpureum
2 Psoralea tenuiflora
3 Ratibida pinnata
1 Silphium integrifolium
1 Silphium terebinthinaceum
Sporobolus heterolepis
Sorghastrum nutans
1 Viola papilionacea
1 Viola pedatifida
13 Zizia aurea

Quadrat #4
(1 meter square)—N. E. Cent.

28 Allium cernuum
1 Amorpha canescens
1 Aster laevis
1 Baptisia leucophaea
24 Coreopsis palmata
1 Eryngium yuccifolium
16 Gentiana puberula
15 Liatris aspera
3 Lithospermum canescens
7 Panicum leibergii
3 Petalostemum purpureum
17 Psoralea tenuifolia
3 Silphium terebinthinaceum
Spartina pectinata
Sorghastrum nutans
Sporobolus heterolepis
4 Rosa carolina
1 Valeriana ciliata

TABLE 2. — Concluded.

Quadrat #5 (*1 meter square*)—*North Cent.*	*Quadrat #6* (*1 decimeter square*)
2 Allium cernuum	
1 Aster ericoides	
6 Aster laevis	
17 Comandra richardsiana	
16 Coreopsis palmata	
1 Dodecatheon meadia	
7 Fragaria virginiana	
3 Hypoxis hirsuta	
2 Liatris spicata	
2 Lithospermum canescens	
2 Pedicularis canadensis	
13 Petalostemum candidum	
83 Petalostemum purpureum	
6 Phlox pilosa	
1 Senecio pauperculus v. balsamitae	
2 Sisyrinchium albidum	
Sorghastrum nutans	
2 Zizia aurea	
	18 Calamagrostis canadensis
	54 Koeleria cristata
	52 Panicum leibergii
	40 Panicum virgatum
	38 Sorghastrum nutans
	18 Spartina pectinata

DISCUSSION

A comparison of the Peacock Prairie with the now destroyed prairie near Elmhurst, Illinois, described by Vestal in 1914, shows that the Peacock Prairie equals or even surpasses the former Elmhurst prairie in richness. It is interesting to note that Weaver (1954) referred to the Elmhurst prairie as "an example of perhaps the most luxuriant type of true prairie".

The presence of the following ten characteristic prairie legumes attests to the richness of the Peacock Prairie:

Amorpha canescens (lead plant)
Baptisia leucantha (white wild indigo)
Baptisia leucophaea (cream wild indigo)
Desmodium illinoense (Illinois tick trefoil)
Lathyrus palustris (marsh vetchling)
Lespedeza capitata (round-headed bush clover)
Petalostemum candidum (white prairie clover)
Petalostemum purpureum (purple prairie clover)
Psoralea tenuiflora (scurfy pea)
Vicia americana (American vetch)

Its richness can also be seen in the presence of two lilies: *Lilium michiganense* (Turk's cap lily) and *Lilium philadelphicum* v. *andinum* (prairie lily); two gentians: *Gentiana andrewsii* (bottle gentian) and *Gentiana puberula* (prairie gentian); and one orchid: *Spiranthes cernua* (ladies' tresses). The Peacock Prairie is the only Cook County station where *Geum triflorum* (prairie smoke) has been reported recently, and it may be the eastern-most station for this plant. It is one of the few prairies in the region that does not contain the ubiquitous *Poa pratensis* (Kentucky blue grass) and *Poa compressa* (Canada blue grass).

While the Peacock Prairie is amazingly rich for its small size, there are a number of prairie species which are found within a twenty-five mile radius

of the prairie but which are not found on it. They include:

Asclepias meadii (Mead's milkweed)
Asclepias viridiflora (green milkweed)
Carex bicknellii (Bicknell's sedge)
Castilleja coccinea (Indian paintbrush)
Ceanothus americanus (New Jersey tea)
Desmodium canadense (showy tick trefoil)
Echinacea pallida (purple cone flower)
Euphorbia corollata (flowering spurge)
Helianthus occidentalis (Western sunflower)
Lespedeza leptostachya (prairie bush clover)
Panicum oligosanthes v. scribnerianum (few-flowered panic grass)
Physostegia virginiana (false dragonhead)
Polygala incarnata (pink milkwort)
Polytaenia nuttallii (prairie parsley)
Rudbeckia subtomentosa (fragrant coneflower)
Salix humilis (prairie willow)
Solidago missouriensis (prairie goldenrod)
Zizia aptera (heart-leaved meadow parsnip)

The lack of the usually common *Euphorbia corollata* on the Peacock Prairie is interesting. This species is found on comparatively rich sand prairies, and its presence in other mesic black-soil prairies may be only due to disturbance. At the same time the high quality of the Peacock prairie is shown by the great variability and lack of uniformity in the vegetative cover. The lack of dominance of any one species, including *Andropogon gerardii* (big bluestem grass), may be an indicator of prolonged virgin conditions.

With little or no management (e.g., fencing, controlled burning, protection from rubbish, vandals) this prairie has been able to maintain itself in comparatively fine condition. Approximately 6 woody plant specimens (all less than two feet high) have established themselves on the prairie. These woody plants belonged to the following species:

Cornus racemosa (gray dogwood)
Populus deltoides (cottonwood poplar)
Prunus serotina (wild black cherry)
Rhamnus cathartica (European buckthorn)
Ulmus americana (American elm)

It is also remarkable that this prairie has been able to repel the invasion of non-prairie plants inasmuch as it is continually being inundated with weed seeds from plants along its edges as well as from the host of exotic trees and shrubs in the surrounding neighborhood.

Apparently some minor disturbance of the prairie may have taken place prior to 1927. The fact that *Achillea millefolium* (yarrow), *Poa pratensis* (Kentucky blue grass), *Antennaria neglecta* (pussy toes) and *Potentilla simplex* (common cinquefoil), which are now either rare or infrequent on this prairie, but which were common and even abundant in some portions of the prairie in 1927 would tend to substantiate this. The minor disturbance could have been due to such factors as trampling by man and by animals, wagon trails, light grazing, and digging prairie

plants for gardens; however, in 1967, there was little evidence of such a disturbance.

At present a number of organizations and individuals are joined together in an effort to preserve the Peacock Prairie for future scientific and educational use. With the almost complete destruction of the eastern tall-grass prairies, the Peacock Prairie should be preserved as a standard for comparison in future ecological work on Illinois prairies.

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FATTY ACIDS OF MARINE OILS AND PIG LIVER LIPIDS

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Abstract. — The total fatty acids and mono-, di- and triglyceride acids of basking shark liver oil were analyzed by gas chromatography of the methyl esters over SE-30 and diethyleneglycol succinate packings. The findings were compared with those of the total acids from two commercial shark liver oil products, raw cod liver oil and carcass lipids of British Columbia salmon, red fish and Norway dog fish in addition to sperm oil and the total and glyceride-derived acids from pig liver lipids. The most prominent acids of the marine oils were 16:0, 16:1, 18:1, 20:1 and 22:1 and the ratio of olefinic to saturated acids was 4.0 but ranged lower for dog fish and sperm oils. The ratio of mono- to polyunsaturated acids was highest for sperm, salmon and Irish pale shark liver oils (4.0-4.7), lowest for cod (1.9) and intermediate for the remaining marine oils. Pig liver fatty acids showed a far greater level of saturated homologs, 16:0, 18:0, 18:1 and 18:2 being the most outstanding components.

Marine lipids in contrast to depot fats of animals, comprise excellent sources of polyunsaturated acids and generally contain relatively low levels of unsaponifiable components (Hilditch and Williams, 1963; Khalid *et al.*, 1968, among others). The iodine numbers of several oils have been correlated with the polyunsaturation of such mixtures by empirical formulas (Ackman, 1966). The polyunsaturated acid moieties of fish oils tend to accumulate in the β-position of glycerol in the glycerides, whereas, marine mammals (whale and seal), not unlike the pig, display primarily shorter fatty acid chains in this position with the longer ones, both saturated and unsaturated, occurring at the α-position (Brockerhoff and Hoyle, 1963; Brockerhoff *et al.*, 1963, 1968).

The present study was undertaken with the view of discerning the composition of the total fatty acids and the component acids of the mono-, di- and triglycerides of the liver oil of the basking shark. The latter species has been investigated in this laboratory with respect to the hydrocarbon, alcohol and sterol constitution, the unsaponifiable portion amounting up to 28% of the oil (Gershbein and Singh, 1968). For comparative purposes, the total acids from saponification of raw cod liver oil and Irish and pale shark liver oils of commerce and carcass lipids of Norway dog fish, British Columbia salmon and ocean red fish as well as sperm oil and pig liver lipids were also submitted to analysis by gas chromatography (GC) over polar and nonpolar packings.

Materials and Methods

All solvents were of AR or CP grade and were distilled prior to use. The basking shark liver oil (Sample M) originated from J. C. Martens & Co., Norway, natural winter sperm oil from Werner G. Smith, Cleveland, British Columbia salmon oil from Shafer-Haggart, Ltd., Vancouver, British Columbia and pale and Irish shark liver oils, North Sea production samples, through the distribution of Arthur Trask Co., Chicago. The latter also made available stock samples of raw cod liver oil and the fish carcass oils. Norway dog and red fish oils, the last one being a Northeastern U. S. product.

Pig liver was obtained fresh from the abattoir and the lipids extracted by blending with methanol, an equivalent volume of chloroform then being introduced. The contents were allowed to stand at 25° C with frequent stirring, filtered and the residue treated with excess 1:1 methanol-chloroform mixture. The filtrate was concentrated under vacuum and the residue taken up in petroleum ether (b. 30-60° C), washed with several portions of water and dried over anhydrous sodium sulfate. Removal of the solvent from the filtrate yielded the lipids in amount of 5% based on the starting tissue.

Total Fatty Acids

The marine oils were saponified by refluxing with 10% aqueous sodium hydroxide for a period of 16 hr. The cooled contents were extracted with portions of ether to remove the unsaponifiable material and the aqueous solution then acidified with sulfuric acid and the fatty acids removed by means of ether. The extract was washed with portions of water, dried over sodium sulfate and the fatty acids obtained by concentration of the filtrate. Hydrolysis of pig liver lipids was also affected by heating with 20% sodium hydroxide in 95% ethanol (Gershbein and Krotoszynski, 1965; Gershbein and O'Neill, 1966).

Separation of Glycerides and Free Fatty Acids

A 4% solution of basking shark liver oil or pig liver lipids in hexane was chromatographed over florisil by the method of Carroll (1961) for the isolation of free fatty acids and the glycerides. The column was eluted successively with the following media, concentration yielding the components as indicated: hexane (hydrocarbons), 5% ether in hexane (sterol esters), 15% ether in hexane (triglycerides), 25% ether in hexane (free sterol), 50% ether in hexane (diglycerides), 2% methanol in ether (monoglycerides), 4% glacial acetic acid in ether (free fatty acids) and methanol (phospholipids). The above procedure was also applied to the isolation of free acids from the Irish shark liver oil. In all cases, the order of distribution was: triglycerides $>>$ diglycerides $>$ monoglycerides.

Esterification of Acids

The free and total fatty acids were esterified by refluxing with methanol saturated with hydrogen chloride. The mono-, di- and triglycerides were transesterified by heating with sodium methoxide in methanol for 30 minutes by the method of Luddy *et al.* (1960). Excess methoxide was neutralized with 30% acetic acid and the methyl esters extracted with hexane, the mixture washed three times with 10% ethanol and the hydrocarbon removed under nitrogen. The methyl esters were analyzed directly as such or after catalytic hydrogenation of aliquots in a Parr low pressure apparatus at 25° C in the presence of Adams' platinum oxide catalyst.

Gas Chromatography

All GC analyses were carried out in a Barber Colman model 5000 gas chromatograph equipped with hydrogen flame detector. The U-shaped glass column measuring 8 ft. x ¼ inch o.d. was charged with 3% SE-30 on 60-80 mesh Gas Chrom P and programmed at 150-280° C, the rate of heating being 2°/min; the detector temperature was 300° C. The carrier gas was helium at 80 ml/min. With 15% diethyleneglycol succinate (DEGS) on 80-100 mesh Gas Chrom P, the column, injector and detector temperatures were 210, 220 and 250° C, respectively; the helium flow rate was 65 ml/min. The methyl esters were dissolved in ether and volumes of 5 μl injected. Tentative assignments for each peak were obtained from a semilogarithmic plot of relative retention time versus chain length and the degree of unsaturation of standard mixtures of methyl esters.

Results and Discussion

The composition of fatty acids obtained by saponification of the liver

oils, fish carcass lipids and sperm oil as based on GC over 15% DEGS is presented in Table 1 and for the total and glyceride acids from basking shark liver oil M, in Table 2. The total acid portions were quite similar in the number and distribution of peaks except for some differences in regard to a few components. Thus, octadecatrienoic acid was not detected in basking shark liver and salmon oils, nor the 20:4 acid in dog fish oil. The 22:2 acid occurred in the latter sample, Irish shark liver and red fish oils, the 22:3 component, in the last product, salmon oil and basking shark liver oil and the 24:1 acid, at 0.9-1.0% levels in basking and Irish shark liver oils but absent or in trace amounts in the other mixtures.

The most prominent acids accounting for at least 70% of the total comprised 16:0, 16:1, 18:1, 20:1 and 22:1. In this regard, hexadecenoic acid ranged lowest in the Irish and basking shark liver oils (2.7 and

TABLE 1.—GC Analyses of Esters of Total Fatty Acids from Fish Lipids and Sperm Oil (DEGS Column)[a]

C-No[b]	Norway Dog Fish	Irish Shark Liver Oil[c]	Pale Shark Liver Oil	British Columbia Salmon	Raw Cod Liver Oil	Red Fish Oil	Sperm Oil
10:0	0.4	0.4 (0.1)	0.1	T[d]	0.2	0.2	0.6
11:0	0.4	0.3 (0.1)	0.1	T	0.2	0.2	0.3
12:0	0.5	0.3 (0.2)	0.2	0.2	0.4	0.2	3.3
13:0	0.3	0.3 (0.1)	0.1	T	0.2	0.2	0.9
14:0	5.2	5.1 (4.2)	3.8	5.9	3.0	4.6	9.0
15:0	0.9	0.6 (0.5)	0.6	0.5	0.8	0.9	4.9
16:0	15.9	11.0 (11.7)	10.3	11.0	12.7	10.6	9.6
16:1	7.2	2.7 (2.4)	5.3	12.8	8.6	7.7	14.9
17:1	0.8	0.7 (0.4)	0.7	0.8	0.9	0.8	1.0
18:0	2.9	2.1 (2.2)	3.2	2.2	2.2	2.1	1.3
18:1	18.8	8.4 (7.0)	20.2	16.0	25.0	15.0	26.5
18:2	1.8	0.9 (0.9)	1.5	1.4	1.8	1.8	1.7
18:3	0.5	0.3 (0.1)	0.3	.....	0.4	0.6	0.9
20:1	10.6	21.5 (21.8)	17.0	18.4	9.3	18.5	8.2
20:2	1.0	0.8 (0.4)	0.8	1.0	1.6	1.0	1.0
20:3	0.5	0.6 (0.2)	0.4	0.6	0.4	0.4	1.0
20:4	.....	0.6 (0.4)	0.8	0.6	1.1	0.4	0.7
22:1	15.9	31.5 (37.5)	18.7	17.0	8.6	15.6	4.6
20:5	4.9	2.7 (2.9)	3.5	4.6	9.8	6.0	2.7
22:2	1.4	0.5 (T)	.....	.....	.....	0.6	.....
22:3	.....	.....	.....	1.3	.....	0.6	.....
24:1	.....	1.0 (T)	.....	.....	T	T	.....
22:4	2.3	3.2 (2.5)	3.3	1.1	2.0	3.3	1.3
22:5	1.9	1.8 (0.8)	1.6	1.1	1.8	2.1	1.0
22:6	5.8	2.7 (3.3)	7.4	3.5	8.9	6.3	4.3
Saturated	26.5	20.1 (19.1)	18.4	19.8	19.7	19.0	29.9
Unsaturated	73.4	79.9 (80.6)	81.5	80.2	80.2	80.7	69.8
Mono—	53.3	65.8 (69.1)	61.9	65.0	52.4	57.6	55.2
Poly—	20.1	14.1 (11.5)	19.6	15.2	27.8	23.1	14.6

a. The total acids were obtained by saponification of the oils.
b. Number of carbon atoms: number of double bonds.
c. Values in parentheses refer to the free fatty acids which were isolated by chromatography of the lipids over Florisil.
d. Trace.

3.3%) and highest in salmon and sperm oils, the levels being 12.8 and 14.9%, respectively. As with the 16:1 acid, octadecenoic acid was lower in basking and Irish shark liver oils, 4.8 and 8.4%, respectively, but otherwise, occurred at 15-26.5% in the other marine oils; the highest levels were displayed by cod liver (25.0%) and sperm oils (26.5%). The 20:1 acid was diminished in dog fish, cod and sperm oils (8.2-10.6%) as contrasted to 17-21.5% for the remaining mixtures. In the case of the 22:1 acid, the lowest amounts were noted in sperm oil (4.6%) and raw cod liver oil (8.6%), maximal levels in basking shark (30.3%) and Irish shark liver oils (31.5%) and intermediate ranges in the remaining products. Octadecenoic acid occurred at 3-9% and was higher in sperm oil (9.0%) and the 20:5 acid ranged from 2.7 to 9.8%, basking shark and cod liver oils containing 8.0 and 9.8%, respectively. A comparable level of the 22:6 acid occurred in the last oil.

The ratio of unsaturated to saturated acids was about 4:1 for the marine products except for greater saturation in the dog fish and sperm oils with values of 2.3 and 2.6, respectively. As would be expected in view of the above discussion, the distribution of unsaturated components also showed variations in type. Thus, the ratios of mono- to polyunsatur-

TABLE 2.—Basking Shark Liver Oil M Total and Mono-, Di- and Triglyceride Fatty Acid Components (DEGS).[a]

C-No.	Total	Mono-	Di-	Tri-
10:0	0.3	.....	.....	.....
11:0	0.3	.....	.....	.....
12:0	0.4	.....	.....	.....
13:0	0.3	.....	.....	.....
14:0	5.8	6.2	6.0	5.2
15:0	0.9	0.4	0.4	0.5
16:0	10.4	14.2	12.7	8.6
16:1	3.3	5.9	5.2	5.1
18:0	1.9	2.1	1.8	1.8
18:1	4.8	7.9	7.2	7.7
18:2	0.9	0.8	0.8	0.9
20:1	19.1	19.6	21.5	20.9
20:2	2.4	2.1	3.0	2.6
20:3	0.4	0.2	0.2	0.3
22:1	30.3	29.7	27.9	32.3
20:4	1.4	0.4	0.2	0.5
20:5	8.0	3.6	4.8	4.9
22:3	1.9	.....	.....	.....
24:1	0.9	.....	.....	.....
22:4	0.5	0.8	0.8	1.2
22:5	0.5	1.0	1.0	0.9
22:6	5.3	4.8	6.2	6.2
Saturated	20.3	22.9	20.9	16.1
Unsaturated	79.7	76.8	78.8	83.5
Mono-	58.4	63.1	61.8	66.0
Poly-	21.3	13.7	17.0	17.5

a. Fatty acids were obtained from the glycerides by transesterification.

ated acids were highest for sperm, salmon and Irish shark liver oils, 4.0, 4.3 and 4.7, respectively, and lowest for raw cod liver oil (1.9); the remaining marine oils displayed values of 2.6-3.2. It should be pointed out that the free fatty acids obtained from Irish shark liver oil by chromatography over florisil simulated the corresponding total acids except for an apparent increase in the monounsaturated homologs (mono-: polyunsaturation, 6.0). Of the two North Sea commercial oils, the Irish shark liver oil more closely resembled the basking shark product in fatty acid composition.

The acids obtained from transesterification of the basking shark liver oil glycerides paralleled those of the total acids in most respects. The octadecenoic acid contents (7.2-7.9%) ranged higher for the glycerides than that of the total acid mixture and the 20:5 acid, lower (3.6-4.9%). The ratios of mono- to polyunsaturated components were 4.6, 3.6 and 3.8 for the mono-, di- and triglycerides, respectively, as compared to 2.7 for the total acid content. However, the ratio of saturated to unsaturated acids was rather constant for the four batches. A comparable distribution of glycerides as well as of the component fatty acids also resulted by application of the thin-layer chromatographic method employing silica gel G to this marine oil (Singh *et al.*, 1966).

In marked contrast to the marine oils, the fatty acids of the pig liver lipids displayed greater saturation, the ratio of olefinic to saturated acids averaging 1.2 for the samples; the ratios of mono- to polyunsaturation were 1.0, 1.5, 1.7 and 1.3 for the total, free, monoglyceride and triglyceride acids, respectively (Table 3). The diglycerides were not analyzed due to overheating of the mixture during processing. The components, 22:0, 22:1 and 22:2 were detected solely in the total acids and the presence of the 22:3 homolog could not be substantiated among the free acids. The most prominent members were 16:0 (16.0-26.7%), 18:0 (12.2-22.5%), 18:1 (25.1-32.0%) and 18:2 (7.7-14.6%). The free and glyceride acids contained more hexadecanoic acid but less octadecenoic acid than the total acid sample and the octadecanoic acid content was lowest for the triglyceride fraction. It will be noted that the last acid made up only 1.3-3.2% of the various marine mixtures (Tables 1 and 2).

Aside from general species differences, the fatty acid composition is greatly dependent on the metabolic and nutritional status of the animal. Such considerations, notwithstanding, the present data are in good agreement with several prior publications, although many of the analyses did not employ GC criteria. In a report by Gelpi and Oro (1968), a South American basking shark liver oil sample showed the presence of about 18.5% each of 18:1, 20:1 and 22:1 acids in addition to 5.5% 12:0, 20.3% 16:0, 6.8% 16:1 and 3.1% 18:0; the total fatty acid content was 33.8%. Such findings are in contrast to the higher acid content of the present product, presumably of north Atlantic origin and containing lower 14:0 and 18:1 and higher 16:1 and 22:1 acid contents, among others. The analysis advanced for British Columbia salmon is at variance with

TABLE 3.—Pig Liver Free and Total Fatty Acids and Acids Derived from the Mono- and Triglycerides (DEGS).

C-No.	Total	Free	Monogly-ceride	Trigly-ceride
14:0	1.1	1.8	1.8	3.0
15:0	0.6	0.9	0.4	1.5
16:0	16.0	20.6	22.2	26.7
16:1	2.0	3.7	2.6	3.8
17:0	0.6	0.4	0.4	0.7
18:0	22.5	20.6	19.1	12.2
18:1	25.1	29.3	32.0	26.7
18:2	14.6	7.7	10.6	9.9
20:0	0.6	0.9	0.8	0.7
18:3	2.3	1.3	1.3	1.5
20:2	0.6	0.4	0.8	1.5
20:3	0.9	0.4	0.8	T
22:0	0.6	.....	.....	.....
22:1	1.1	.....	.....	.....
20:4	4.1	7.8	4.0	3.8
22:2	2.0	.....	.....	.....
22:3	1.4	.....	T	0.7
22:4	1.4	0.9	0.4	3.8
22:5	1.1	1.3	1.3	1.5
22:6	0.9	1.3	0.8	1.5
24:0	T	T	T	T
Saturated	42.0	45.2	44.7	44.8
Unsaturated	.....	.....	.....	.....
Mono-	28.2	33.0	34.6	30.5
Poly-	29.3	21.1	20.0	24.2

data reported for Coho salmon (Saddler *et al.*, 1966), the former showing higher 20:1 and 22:1 contents and diminished 18:2, 20:3, 20:4 and 22:6 levels. However, aside from the species differences, Stansby (1967) demonstrated wide variations in the fatty acid composition of Coho salmon from fresh and salt water and for fingerlings.

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SENSORY CANALS OF THE SNOUT OF ACTINOPTERYGIAN FISHES

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Abstract. — The snout of the earliest palaeoniscoids is more modified in terms of developmental facts than is that of later actinopterygian fishes. This specialization demands that later fishes, presumably derived from the palaeoniscoids, arrived at their condition by neoteny and functional reorganization of snout elements. Inaccuracies in accounts of snout structures are also reported.

Recently Gardiner (1963) reconsidered some ideas concerning the structure of the actinopterygian snout. In his opinion the primitive condition was one of extreme shortness associated with a relatively large eye (Fig. 1A). He stated that the primitive paleoniscoid fish had two nasal openings and that these were widely separated: the anterior opening lying between the "postrostral" and the nasal bone, and the posterior opening behind the nasal bone at the anterior margin of the eye. The supraorbital canal connected with the infraorbital canal by way of a short branch of the latter and this connection passed between the nasal openings. He proposed (p. 315) that "In order to arrive at the condition seen in both holosteans and teleosts, with regard to the nostrils, it is necessary to get both of the nostrils to the lateral side of the supraorbital sensory canal." And (p. 317), ". . . in *Polypterus senegalus* we have the answer to the problem, that is that the anterior nostril migrated round the anterior edge of the nasal (bone), passing round the contained supraorbital sensory canal to end up on the posterior side of the nasal." (Fig. 2).

It is not my purpose to take issue with what Gardiner has said but rather to discuss the canal pattern as seen in several of the living "primitive" actinopterygians and teleosts and suggest that what has occurred is not well described by the term "migration"; it is more like reorganization. And, if reorganization has occurred, there might be some question as to the original state. As background for an approach to these questions, I want first to consider (briefly) the ontogeny of the nasal capsule and the lateral-line system and then the adult canal-nostril conditions.

The development of the nasal organ is generally agreed to involve an epidermal placode which invaginates. The external opening of the vesicle, at a relatively early stage, subdivides into anterior and posterior openings which quickly assume the position of the adult apertures. This

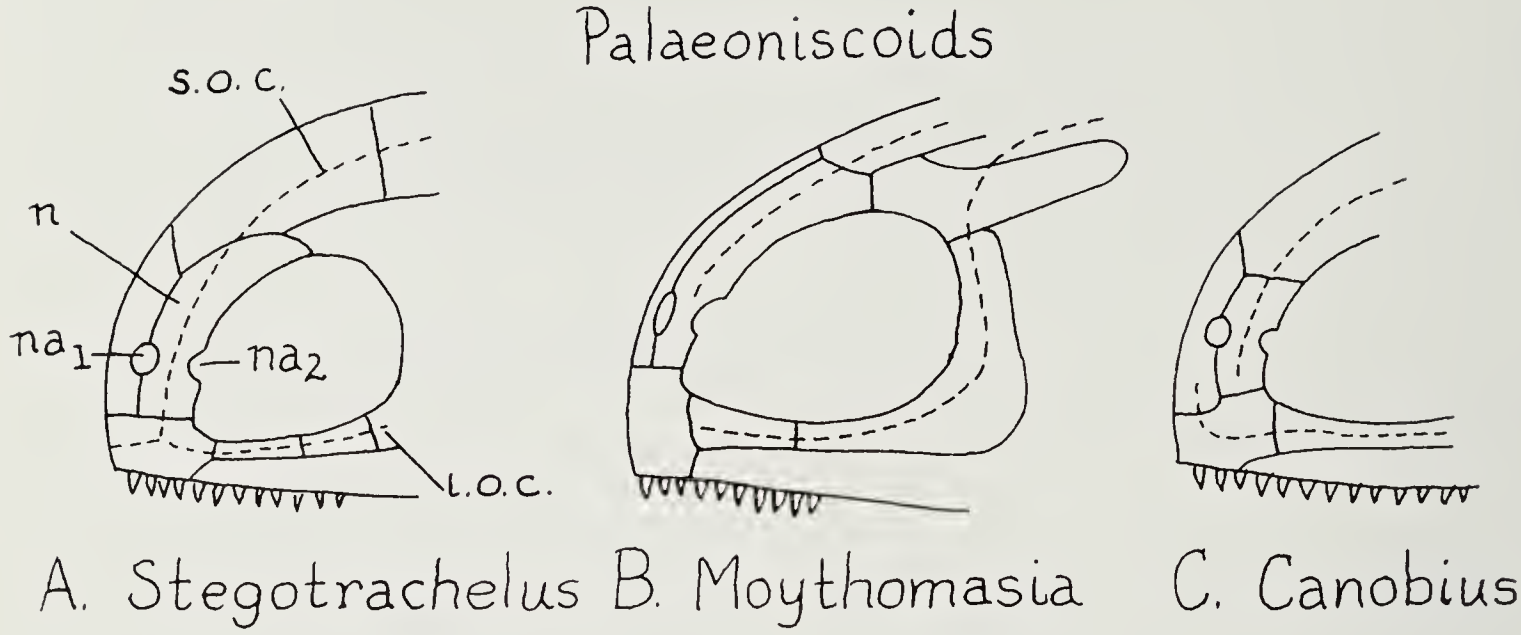

FIGURE 1. — Semidiagrammatic lateral views of the snouts of early palaeoniscoids (A, B, C) and holosteans (D, E). *n*, nasal bone; na_1, anterior nasal opening; na_2, posterior nasal opening; *m.r.*, medial rostral; *l.r.*, lateral rostral; *i.o.c.*, infraorbital canal; *s.o.c.*, supraorbital canal; *x*, unnamed bone. A, D, E. after Gardiner, 1963; B. after Jessen; C. after Moy-Thomas and Dyne, 1938.

view of the origin of the two nasal openings in fishes is uncontested. The relationship between the nasal vesicle and the mouth cavity (and Rathke's pouch) is not a part of this discussion.

Development of the lateral-line system is a more complex subject. Pehrson (1958, 1940) has considered this system in *Polypterus* and *Amia*, as has Hammarberg (1937) for *Lepisosteus* and Lekander (1949) for a number of teleosts. Several basic works on the comparative anatomy of the lateral-line system have appeared (Stensiö, 1947; Holmgren and Pehrson, 1949).

The lateral-line system appears first as a series of epidermal placodes in the postorbital and temporal region. The supraorbital and infraorbital placodes grow forward above and below the eye and subdivide into a variable number of maculae. The number of maculae is high in *Lepisosteus* (20-24 in the supraorbital line and anterior pit-line, 20-25 in the infraorbital), lower in *Amia* (6 or 7 in the supraorbital plus 2 in the anterior pit-line and 14 in the infraorbital), *Polypterus* (6 in the supraorbital, 8 in the infraorbital), and salmon (*Oncorhynchus nerka* — 8 or more in the supraorbital, 12 more-or-

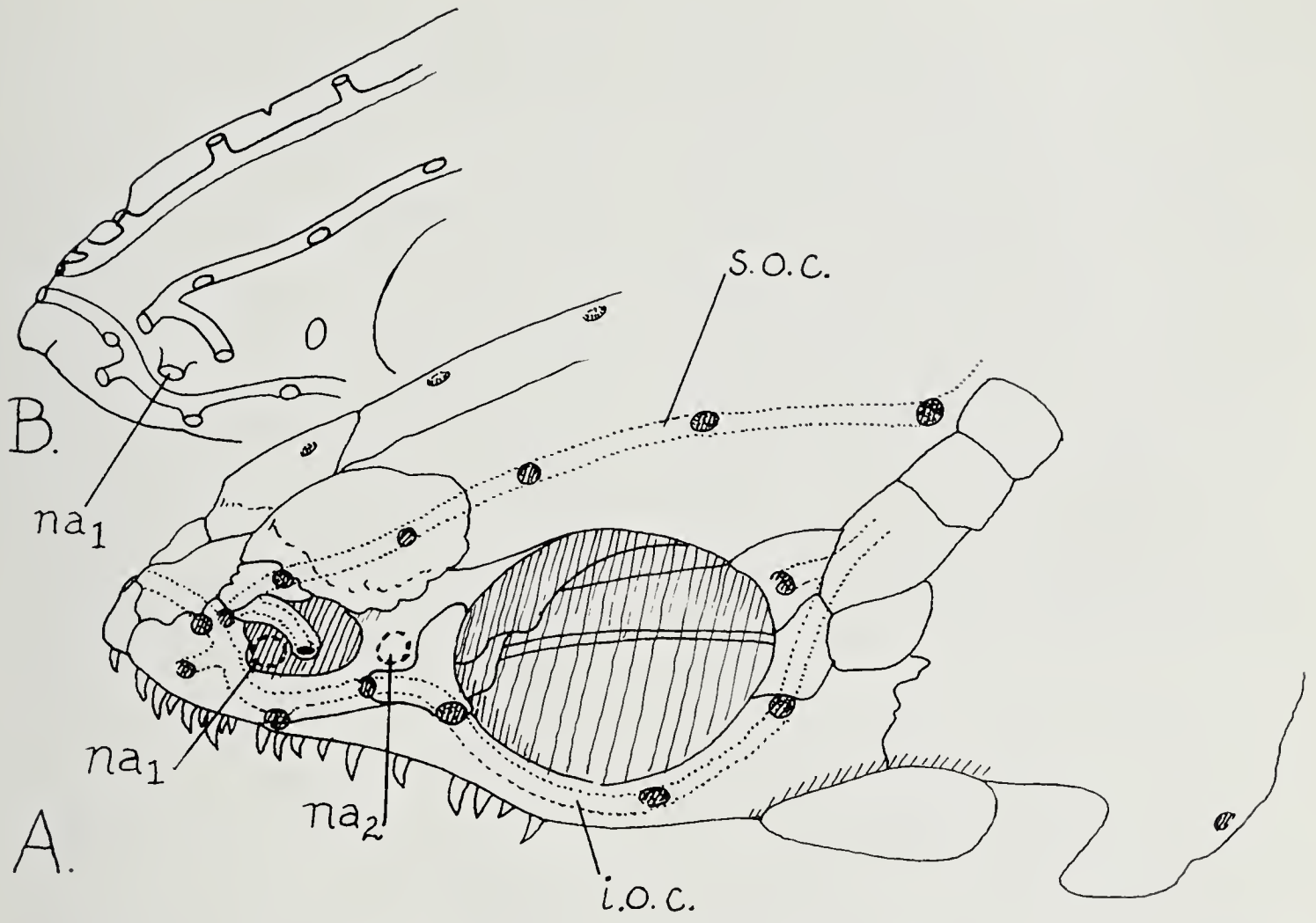

FIGURE 2. — Dorsolateral views of snout of *Polypterus* (*weeksi*?). A. Bones, canals and pores; B. details of bones removed. Abbreviations as in Fig. 1.

less in the infraorbital, and 2-3 which subdivide in the snout pit-line).

In development the maculae, as small epidermal placodes, sink down into the underlying tissues, first forming a pit, then a groove (along the course of the original sensory line placode), and then a looping canal with ends opening at the surface. The macula lies at the deepest point of the U-shaped canal thus formed. These canal units (each with its separate organ) then join one with another to form a continuous canal but retain a surface pore where adjacent ends have joined. The surface pores may later divided to form complex dendritic systems.

Some sensory organs may remain at the surface in grooves, or in pits, to form the pit-lines and these may be continuous with, or separate from, the canals. Part of this system, normally forming canals, may develop only to the pit-line stage. As a generality one can say that any connections achieved anteriorly between the supraorbital and infraorbital canals are secondary ones, as are connections across the midline between any of the canals.

Comparative study of sharks, dipnoans, crossopterygians and actinopterygians shows that the relationship between the supraorbital canal and the infraorbital canal varies as does the relationships between these lines and the nasal openings (Fig. 3). Only in the crossopterygians does the supraorbital canal connect with the infraorbital canal medial to the anterior nasal opening, while in actinopterygians, as suggested by Gardiner, the connection may have been be-

tween the two openings. In sharks the connection is behind the nasal capsule. The dipnoans are unique in several ways (Panchen, 1967): the two canals in question do not appear to join; the supraorbitals connect across the snout (as in holocephalans); and both canals pass above and lateral to the olfactory openings (as in holocephalans, but the infraorbital canals do not meet at the midline as in that group).

Because of the known variation in relationships in the actinopterygians, the snout in this group should be rechecked. In order to do this the adult condition and some of the developmental stages of *Polypterus, Acipenser, Polyodon, Lepisosteus,* and *Oncorhynchus* (*O. nerka, O. kisutch* also *Salmo gairdneri, Salmo trutta, Salvelinus frontinalis, Salvelinus namaycush*) are redescribed. Of the above, only *Amia* is represented by a more-or-less full set of stages, *Lepisosteus* and *Oncorhynchus* by a sample of stages, and the others are known only as adults or from accounts in the literature. *Polypterus* is considered first since Gardiner

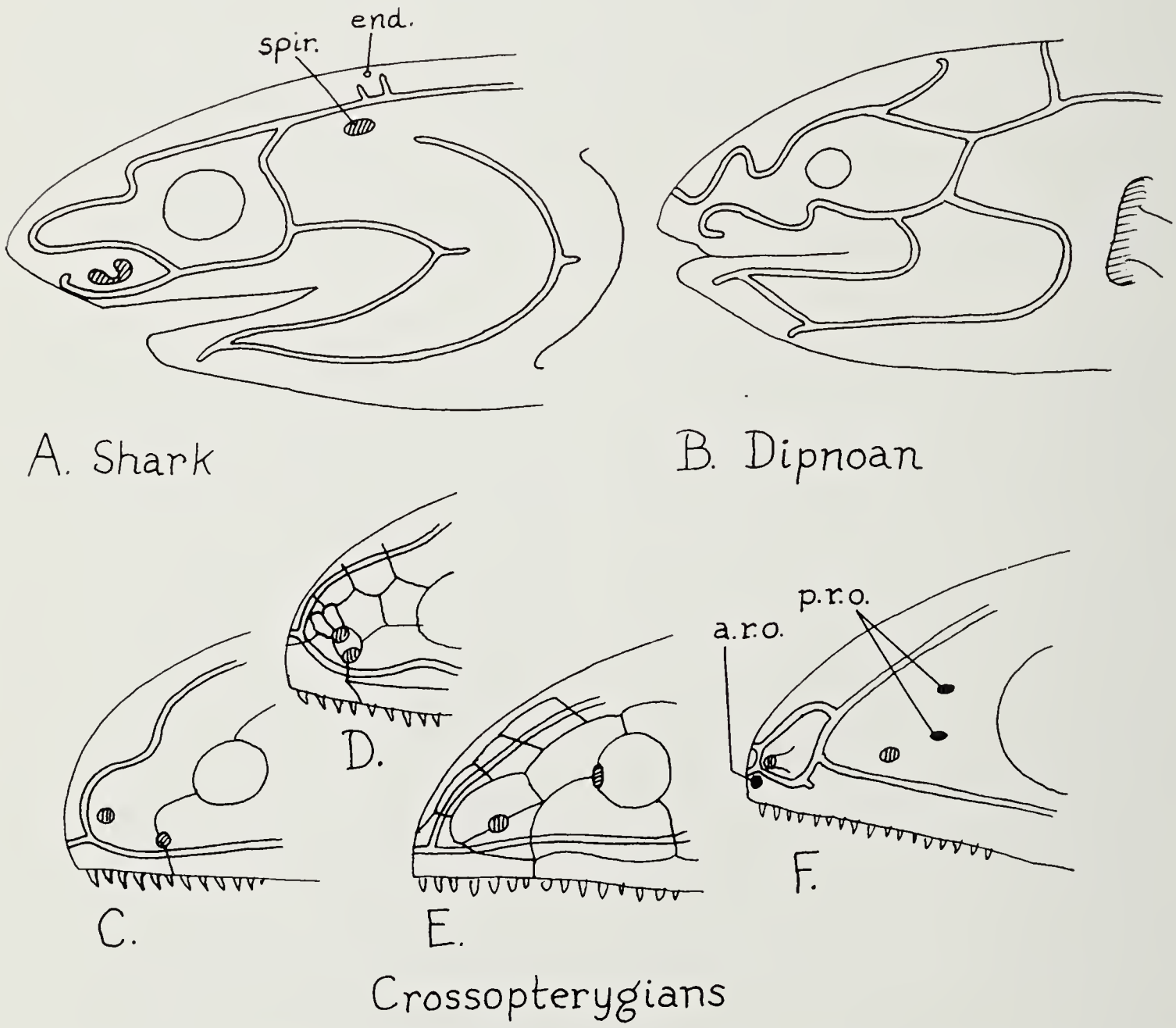

FIGURE 3. — Lateral views of heads and snouts of A. *Chlamydoselachus*; B. *Protopterus*; C. *Porolepis*; D. *Holoptychius*; E. *Eusthenopteron*; F. *Latimeria*. *end.*, endolymphatic duct pore; *spir.*, spiracle; *a.r.o.*, anterior rostral organ opening; *p.r.o.*, posterior rostral organ opening; others as in Fig. 1. A. modified from Allis, 1923; C, D, E. after Jarvik, 1966; F. after Millot and Anthony, 1958.

viewed this as a transitional stage in the shifting of the nostril relationship.

In my three specimens of *Polypterus* (*weeksi*?) (Fig. 2) the penultimate pore of the supraorbital canal surfaces in the bone next to a pore of the infraorbital canal. These two tubes join to form a single opening through the overlying skin. As suggested by Gardiner, this is a secondary condition. However, from here the supraorbital canal is reflexed posterolaterally (as remarked by Jarvik, 1942, and Pehrson, 1958) to end at a surface pore behind the base of the tube of the anterior nasal opening. I question that there has been migration of the anterior nasal opening; rather there has been a looping of the supraorbital canal to achieve a new connection medial to the anterior opening.

In *Lepisosteus*, contrary to Gardiner's account, the supraorbital canal (Fig. 4A) connects with the infraorbital between the narial openings as well as behind them. These connections are by well-defined canals in the soft tissue, canals which open to the surface by many small pores (achieved by subdivision of the original single opening).

The canal connection between the nasal openings can be compared with the one seen in palaeoniscoids, while the posterior one is probably a special feature associated with the long snout. Both connections involve the lateral rostral element. The reflexed anterior end of the supraorbital canal compares with that seen in *Polypterus* but differs in that a connection medial to the anterior nasal opening is not developed (even by way of pores to the surface).

In *Amia* (Fig. 4B) a connection between the supraorbital and infraorbital canals is developed relatively late in ontogeny (200 mm specimen). This connection passes between the nasal openings but is not comparable to that of the palaeoniscoid as the greater part of the canal is an upward extension of the infraorbital canal on the lateral rostral bone. As in the preceding genera, the anterior end of the supraorbital canal is turned lateroposteriorly and as in *Polypterus* ends at a pore above the base of the anterior nasal tube. Again no con-

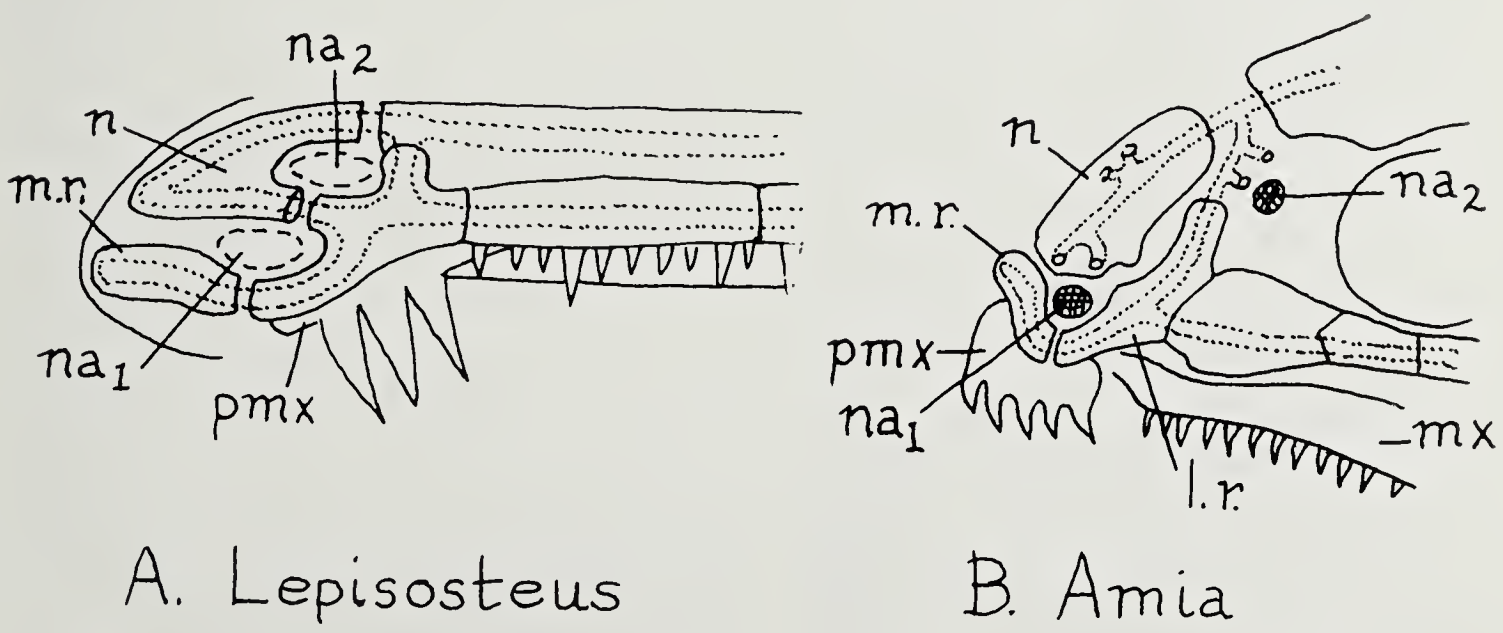

FIGURE 4. — Semidiagrammatic views of snouts of *Lepisosteus* (A) and *Amia* (B). Note differences when compared with Fig. 1, D and E.

nection exists medial to the anterior nasal opening although there is a relatively long tube to the surface here.

Thus in these genera, the anterior end of the supraorbital canal is turned laterally or posterolaterally; however, a sutural connection between the nasal and rostral bones is lacking (although Gardiner, footnote p. 317, described a bridge as "occasional" in *Lepisosteus*). In both *Amia* and *Lepisosteus* a lateral rostral bone, with an upward extending canal, is distinctive. Another important point here is that the commonly used illustrations in the literature (as in Gardiner, *see* Fig. 1 D, E) contain inaccuracies relative to the interrelationships of these canals or the bony cover of the snout. In *Amia* the bones are not sutured but separated by connective tissue. Further such figures conceal other facts such as that the "premaxillae" of these fishes are distinctly different. This difference runs much deeper than whether or not canal bones are fused to the premaxilla.

The canal situation seen in these fishes is in part shared with other living kinds. In *Acipenser* (or *Scaphirynchus* — Grassé 1958, fig. 696; Norris, 1924, 1925) a bony tube lies in the tissue between the anterior and posterior nasal openings. This tube can be compared with that seen in *Polypterus*; its canal does not connect with the intraorbital, nor does the canal continue much beyond this bone (as shown in Goodrich, 1930, fig. 298; Gardiner, 1963, fig. 20A; or in *Polyodon* as shown by Collinge, 1894, pl. 39, fig. 3). The course of the supraorbital canal in the chondrosteans is that of a palaeoniscoid, however, the lateral rostral bone, where connection between the supraorbital line and the infraorbital line might occur, is near the tip of the elongated snout (Pehrson, 1944, and personal observation). The snout with its transverse connection of the infraorbital canals develops early in the sturgeons, before the extension of the supraorbital canal between the nasal openings.

In *Oncorhynchus* (*Salmo* or *Salvelinus*) the supraorbital canal ends above the nasal sac and the infraorbital below that sac. There is a surface pit-line continuation of the infraorbital canal upward in front of the anterior nasal opening and across the snout. The pit-lines of either side appear to join at the midline. The supraorbital canal does not have any definite relation to this pit-line (it may open into the groove of the pit-line). In the teleost *Elops* (Nybelin, 1956) the infraorbital canals connect across the snout but the supraorbital canal, lying above or medial to the nasal sac, does not connect with the infraorbital. There is no clear evidence in these teleosts of the supraorbital canal bending laterally or posterolaterally to pass between the nasal openings, although in early stages of the salmon there is a suggestion of such a bend. An infraorbital branch extends upward on the lateral rostral (antorbital) bone in *Elops* but not in the salmon where only a groove is sometimes observed.

It is evident that some of the teleosts (it is difficult to talk about such a group since it is as yet only poorly understood and extremely heterogeneous anatomically) differ from the more primitive genera in that the sensory canals open to the surface by large pores, and in that the develop-

ment of the nasal capsule proceeds only to a slight separation of the anterior and posterior nasal openings. From the ontogeny of *Amia* (Allis, 1889; Pehrson, 1922, 1940) it can be said that the lack of separation of nasal openings and lack of subdivision of the surface pores are neotenic trends or secondary simplifications. Reduction of canal development is carried to the extreme in *Cobitis taenia* in which the sensory organs remain on the surface (Lekander, 1949). The forward growth of the supraorbital canal medial to the nasal openings, without forming a loop, suggests a secondary condition also. Failure to connect with the infraorbital canal cannot be viewed as exceptional.

In Gardiner's presumed primitive palaeoniscoid the supraorbital canal traversed the nasal bone and joined, or nearly joined, the infraorbital canal (Fig. 1A-C). He suggested that primitively these canals joined, but that this connection was later lost and the ends of the canals shifted apart. The area of connection of these canals should be that of the lateral rostral bone although such an element is evident only in a few palaeoniscoids.

If the sequence suggested by Gardiner is correct, we must assume that the nasal capsule and lateral-line system were well established and highly evolved structures when first encountered in the fossil record, not in the process of being developed, as one might suppose. These advanced features are associated with a well-defined cranial pattern of bones — again the argument that in these fishes the pattern of bones was in the course of development from a mosaic seems improbable. The modified conditions seen in other osteichthyan fishes and sharks (Fig. 3) suggest that they too are not primitive. The several patterns observed in the osteichthys imply an early radiation involving snout design. Romer (1958) made the comment that actinopterygian fishes were eye feeders and crossopterygians nose feeders. From these observations, there is the possibility that internal nares were common to all osteichthys at the beginning. Modification of the primitive nasal set-up was thus greatest in the dipnoans and most conservative in the rhipidistian crossopterygians. Presumably the gap between the premaxillary and maxillary teeth (and bones) marks the pathway of the primitive internal nasal opening.

Gardiner has supposed that primitively there was a large number of sensory organs, as in sharks (supraorbital canal in *Squalus*, 68; *Laemargus*, 80+; *Heptanchus* and *Chlamydoselachus*, 46-48 according to Ewart, 1895; Ewart and Mitchell, 1895; Norris and Hughes, 1920; Daniel, 1934; Allis, 1923) or acanthodians. Thus strong reduction in the number of organs was assumed to have marked the common ancestor of the teleostomes, or occurred in parallel fashion in each of the teleostome groups. It is probable that the primitive condition, before the origin of the osteichthys, was characterized by a large number of organs, some of which were involved in the canal system and condensed to form the larger sensory organs of the ancestral palaeoniscoid. The large number of organs in sharks may be a part of their evident regression related to the

loss of bony armor and its replacement by the fine placoid shagreen.

What we see in the later evolution of the actinopterygians is in each case a different set of features; some of the components can be viewed as neotenic while others appear to be reorganizational or functional refinements. The snout appears to lengthen in some groups while the relative size of the eye is decreased. These structural changes can be related to changes in the feeding mechanism and some parallelisms are evident.

In summary there is no evidence to support the use of "migration" in reference to either or both nostrils, and certainly there is no evidence of movement of a nostril around the end of the supraorbital canal or "nasal" bone. What is involved is best described as ontogenetic reorganization. The relationship of the supraorbital canal to the nostrils in chondrosteans, *Amia*, *Lepisosteus*, and *Polypterus* supports Gardiner's view that originally this canal passed between them, whether or not it connected with the infraorbital canal. Any connection between the supraorbital and infraorbital canals would necessarily be formed in ontogeny after the separation of the original nasal opening into anterior and posterior nares. If this was the condition in the ancestral actinopterygian, it was not a primitive one but rather an evolved state which later regressed in the evolution of the group. All trace of the route of the supraorbital canal between the nasal opening has been lost in some of the living fishes and in others new secondary connections between the canals may be formed medial to the anterior naris, or elsewhere. These changes probably can be tied in with lengthening of the snout, increased kinetism in the snout, reduction in relative size of the eye, and the developmental dynamics of the canal system.

In terms of the snout the earliest palaeoniscoid appears to have been an armored group already specialized in terms of its nasal apparatus and lateral-line system. Some of the later palaeoniscoids and living fishes must have been derived either from a pre-palaeoniscoid ancestor in which these features had not yet developed or, more probably, from the palaeoniscoids by reduction in armor and remodeling of the various snout components and features.

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THE FISHES OF PIASA CREEK, WEST CENTRAL ILLINOIS

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ABSTRACT. — A survey of the fishes of Piasa Creek, in Jersey, Madison and Macoupin counties, west central Illinois, included 41 collections from 31 sites totaling 23,475 specimens of 44 species. The fauna consists of: 1) river and large water species found only in the lower portions of the drainage, 2) ubiquitous small stream species of the area, 3) isolated populations of *Chrosomus erythrogaster* and *Cottus carolinae* in the upper reaches of Mill Creek.

Piasa Creek and its four tributaries drain approximately 150 square miles in Macoupin, Jersey and Madison counties in west central Illinois, and is the only major tributary entering the Mississippi River from Illinois between the mouths of the Missouri River and the Illinois River. The mouth of Piasa Creek is 14 miles north of the mouth of the Missouri River and 9 miles south of the Illinois River. Piasa Creek proper flows essentially north-south with the upper portion of the drainage forming a dendritic drainage pattern in soft Pennsylvanian sandstones and shales (Figure 1). The lower portion of the creek is on more resistant Mississippian limestones and a trellis drainage is developed following joint lines parallel to the strike of the Lincoln anticline. Approximately one mile upstream from the mouth, Mill Creek joins the main creek from the west, and four miles further upstream Rocky Fork joins from the east. These two tributaries flow through the Mississippian limestones and thus typically have rock and gravel bottoms. The upper two tributaries are both called "Little Piasa Creek." The lower joins from the east approximately eight miles above the mouth and the upper from the west two miles further upstream. The upper main creek and the upper reaches of these two tributaries flow through sandstones and shales and typically have sand and mud bottoms.

Piasa Creek was not included in the localities cited by Forbes and Richardson (1908) but is of considerable interest because of its unique location. During this study, 41 collections were made at 31 sites and a total of 23,475 specimens representing 44 species were taken. Of particular interest is the discovery of isolated populations of the southern redbelly dace, *Chrosomus erythrogaster*, and the banded sculpin, *Cottus carolinae*, in the upper reaches of Mill Creek.

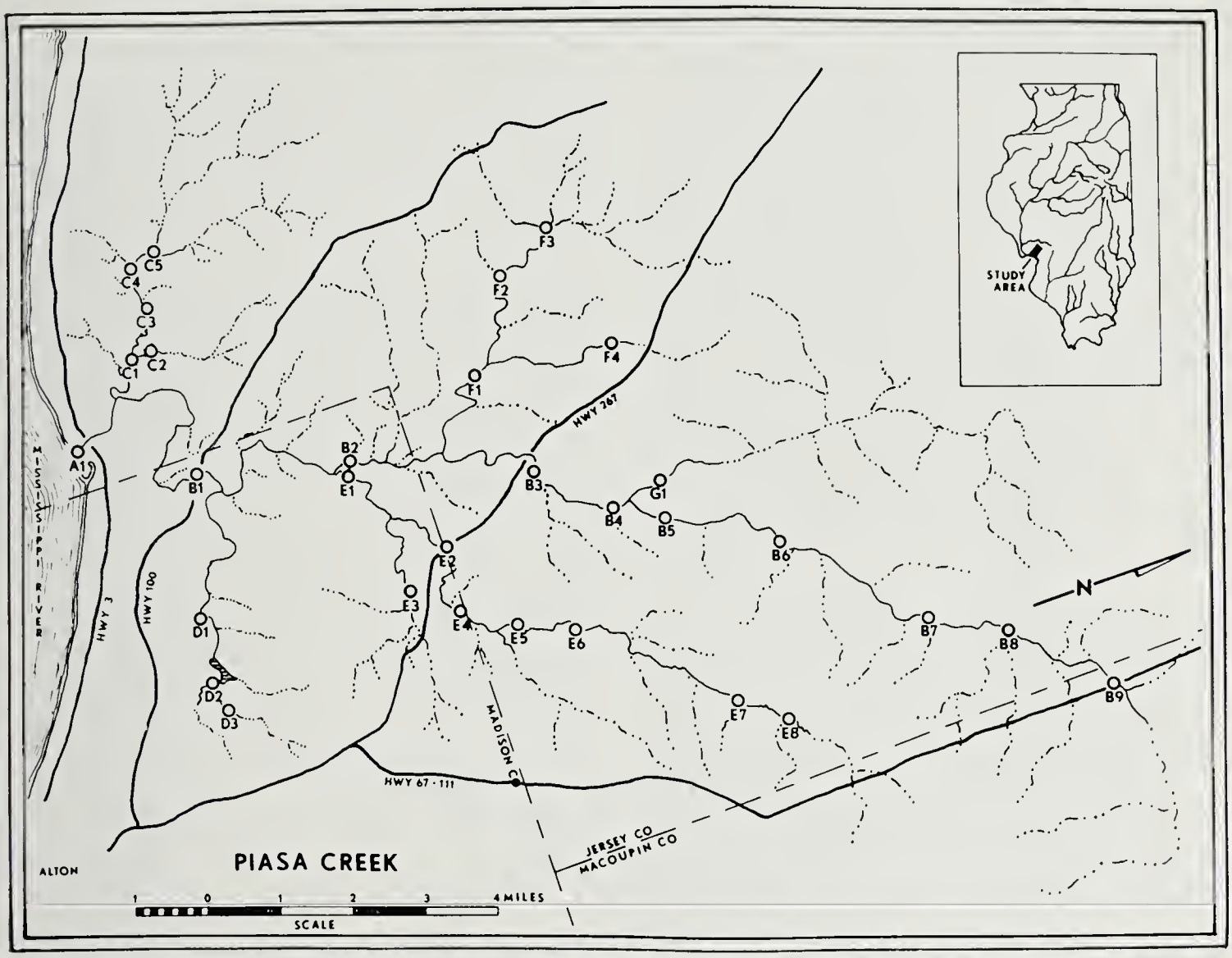

FIGURE 1. — The Piasa Creek system in west central Illinois. Collection sites marked with open circles. Sites A1, B1-B9 on Piasa Creek, sites C1-C5 on Mill Creek, D1-D3 on Rocky Fork, E1-E8 on "Little Piasa East", F1-F4 on "Little Piasa West", G1 on unnamed tributary of Piasa Creek.

METHODS AND MATERIALS

Most of the fish studied were collected by Smith and Twillman between 7 May 1967 and 23 August 1967. Five other collections made by classes at Southern Illinois University are included in the data. Previous to this study two collections totaling 198 specimens of 19 species had been deposited at the Illinois Natural History Survey (INHS). These two collections are listed under the site accounts but are not included in our data. All collections other than these two are deposited at the Southern Illinois University at Edwardsville (SIUE). Collections were made with 6, 10, and 15 by 6 foot woven nylon seines during daylight hours with a few exceptions. As noted in the site accounts a few collections were made with a 30 by 8 foot bag seine or at night. These methods did not adequately sample the lower portion of the main creek (A1 and B1) but we feel our samples are representative of the fish populations present at the rest of the sites.

COLLECTION SITES

The 31 sites collected are described below. Included are pertinent data pertaining to the collections made at each site. Each site is identified by letter and number (Figure 1) and located by longitude and latitude. Many are on unnamed roads but common locations are given where possible. The date of collection(s) follows the locality data. Total number of fishes taken is given in parentheses after the collection date. At sites which were collected more than once, collections are listed in chronological order and any unique conditions are given for each collection. Unless otherwise noted collections were made by Smith and Twillman.

SPECIES	SITE																													
	A 1	B 1	B 2	B 3	B 4	B 5	B 6	B 7	B 8	B 9	C 1	C 2	C 3	C 4	C 5	D 1	D 2	D 3	E 1	E 2	E 3	E 4	E 5	E 6	E 7	E 8	F 1	F 2	F 3	F 4	G 1
Dorosoma cepedianum	A	P	...	R	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Carpiodes cyprinus	...	P	...	P	R	P	R	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Catostomus commersoni	...	P	...	C	...	...	R	C	P	...	R	...	...	C	R	...	...	...	R	P	...	...	...	...	...	...	C	C	R	...	P
Campostoma anomalum	...	...	P	C	C	C	C	C	C	...	P	A	R	A	C	...	...	...	A	A	C	P	C	A	A	A	A	A	A	A	C
Chrosomus erythrogaster	...	...	...	...	...	...	...	...	...	...	...	...	P	A	A	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Hybognathus nuchalis	...	P	...	R	R	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Notemigonus crysoleucas	P	R	...	...	R	...	...	...	...	...	...	...	...	R	...	A	P	...	...	R	...	...	...	...	...	...	...	...	...	...	...
Notropis atheridinoides	C	A	...	...	...	...	...	...	...	...	A	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
N. dorsalis	...	A	A	A	A	A	A	A	A	...	P	P	P	C	C	P	...	A	A	A	...	A	A	A	C	C	A	A	C	C	A
N. lutrensis	...	A	...	C	C	...	R	R	R	...	A	P	C	P	R	C	...	...	...	C	...	...	...	...	...	...	R	P	...	A	...
N. stramineus	...	A	C	C	C	P	R	R	R	...	...	...	C	P	...	...	...	...	C	C	...	...	...	...	...	...	R	...	...	...	P
N. umbratilis	...	R	...	P	P	...	...	R	...	...	C	P	A	C	...	R	...	...	...	P	...	...	...	...	...	...	...	...	...	...	...
Phenacobius mirabilis	...	R	...	R	...	...	...	R	R	...	...	...	R	...	...	...	...	...	...	R	...	...	...	...	...	...	...	...	...	...	...
Pimephales notatus	...	C	P	P	P	...	...	R	...	...	A	P	A	C	C	P	...	...	...	A	...	...	...	...	...	...	P	R	R	P	...
P. promelas	...	P	...	R	P	...	...	...	...	A	R	...	...	R	...	C	...	...	...	P	...	...	...	...	...	...	R	P	R	R	...
Semotilus atromaculatus	...	C	P	C	C	C	A	A	A	...	P	A	P	C	C	P	A	...	P	A	A	P	C	A	A	A	A	A	C	C	A
Ictalurus melas	...	R	...	R	...	...	...	...	...	R	C	R	...	...	...	...	...	...	...	R	...	R	...	...	P	R	...	...	...	P	...
I. natalis	...	...	...	R	R	...	...	...	R	...	P	...	P	R	R	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Fundulus notatus	...	C	P	P	...	...	...	...	...	...	C	...	P	P	...	...	...	...	...	R	...	...	...	...	...	...	...	...	...	...	...
Lepomis cyanellus	P	P	...	R	R	...	...	P	R	A	P	...	P	C	P	P	...	...	...	P	...	R	R	P	P	...	R	R	...	C	R
L. humilis	A	R	...	R	R	...	...	...	...	...	...	...	...	...	...	...	...	...	...	P	...	...	...	...	...	...	...	...	...	...	...
L. macrochirus	A	P	P	R	C	...	...	...	...	...	P	C	P	...	...	C	C	...	...	C	...	R	...	...	P	...	...	...	...	...	R
Micropterus salmoides	...	R	...	R	R	...	...	...	...	...	R	...	...	...	...	...	...	...	...	R	...	...	...	...	...	...	...	...	...	...	...
Etheostoma spectabile	...	P	C	C	P	C	C	C	A	...	...	R	R	C	P	...	...	...	C	C	...	C	C	C	A	P	A	P	P	P	C
Cottus carolinae	...	...	...	...	...	...	...	...	...	...	...	...	...	P	P	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE 1.—Relative abundance of common species at 31 collecting sites on Piasa Creek. A=Abundant above 20%, C=Common 5-20%, P=Present 1-5%, R=Rare under 1%.

Piasa Creek

A1: 38° 56′ 10″ N — 90° 17′ 12″ W, Piasa Creek Public Boat Ramp off Highway 3, 100 yards upstream from mouth; 5-17-66, (62), Thomerson and ichthyology class, bottom soft mud, many snags, width 100 to 150 feet, only shoreline seined.

B1: 38° 57′ 27″ N — 90° 16′ 15″ W, Piasa Creek and Highway 100; 4-5-66, (315), Thomerson and ichthyology class, water clear and white, current swift, bottom soft sand and gravel, width 5 to 20 feet, depth to 5 feet; 5-17-66, (138), Thomerson and ichthyology class, water turbid white, current swift, bottom sand and gravel, width 100 feet, depth to 6 feet, water high and hard to seine; 5-19-66, (27), Grover, Osborne, and Whipple, water turbid, bottom sand, gravel and mud, width 75 feet depth to 6 feet; 6-1-66, (70), O. M. Price (INHS); 7-11-67, (519), water slightly turbid, bottom sand and mud, width 10 to 20 feet, depth to 5 feet, some algae present; 8-23-67, (772), Smith, Twillman, Thomerson and McAffee (30 foot bag seine), water muddy, current flowing upstream, bottom sand, gravel and mud, width 8 to 20 feet, depth to 5 feet.

B2: 38° 59′ 15″ N — 90° 15′ 35″ W; 6-14-67, (143), water clear, bottom sand, width 5 to 15 feet, depth to 2 feet, pH 8.0, DH 23°.

B3: 39° 01′ 15″ N — 90° 14′ 30″ W; 8-1-63, (128), Braasch and P. W. Smith (INHS); 7-5-67, (1212), water clear, bottom sand, width 3 to 15 feet, depth 3 feet; 7-27-67 (Night, 2015-2115), (1249), water muddy, bottom sand and mud, width 4 to 15 feet, depth 3 feet; 9-29-67, (2379), Thomerson, Smith, Twillman and class, water slightly turbid white, bottom sand and silt, width 3 to 20 feet, depth to 3 feet. The same species were taken by day and night but about three times as many fish were taken with the same effort at night.

B4: 39° 01′ 58″ — 90° 13′ 40″ W; 7-5-67, (284), concrete ford creates a pool above ford, water clear, bottom mud and sand, width 3 to 15 feet, depth to 3 feet.

B5: 39° 02′ 30″ N — 90° 13′ 12″ W; 7-5-67, (278), water clear, bottom sand, width 2 to 5 feet, depth to 1 foot, mainly riffles.

B6: 39° 03′ 30″ N — 90° 12′ 20″ W; 7-27-67, (912), water clear, bottom sand, width 4 to 10 feet, depth 1 to 3 feet, water temperature 26° C.; some algae present.

B7: 39° 05′ 00″ N — 90° 10′ 23″ W; 7-27-67, (411), water muddy, bottom mud and gravel, width 6 to 10 feet, depth to 4 feet, water high due to rain, some algae, pools and riffles.

B8: 39° 05′ 50″ N — 90° 09′ 50″ W; 7-27-67, (674), water muddy, bottom sand and gravel, width 3 to 10 feet, depth 3 feet, pool and riffles.

B9: 39° 06′ 50″ N — 90° 08′ 30″ W; Highway 111 and Piasa Creek; 7-27-67, (127), water muddy, bottom mud and sand, width to 20 feet, depth to 4 feet, water 1 foot above normal. *Lepomis cyanellus* was dominant species.

G1: 39° 02′ 40″ N — 90° 13′ 45″ W; 7-5-67, (633), water clear, bottom sand, width 5 feet, depth 1.5 feet, on small side branch.

Mill Creek

C1: 38° 57′ 10″ N — 90° 18′ 15″ W; 7-29-67, (108), water muddy, bottom mud, width 10 feet, depth to 6 feet, water high, current reversed; 8-23-67. (279), Smith, Twillman, Thomerson and McAffee, water slightly turbid, bottom sand, width 10 feet, depth to 3 feet.

C2: 38° 57′ 27″ N — 90° 18′ 16″ W; 7-29-67, (128), water muddy, bottom mud, width 10 feet, depth 3 feet, a single pool under bridge.

C3: 38° 57′ 01″ N — 90° 18′ 68″ W; 5-7-67, (122), water muddy, bottom sand, gravel and mud, width 3 to 5 feet, depth to 3 feet, pools and riffles.

C4: 38° 57′ 05″ N — 90° 19′ 55″ W; 5-7-67, (250), water muddy, bottom sand, gravel and mud, width 10 to 15 feet, depth to 4 feet, pools and riffles; 7-27-67, (1154), (night 2245-2330), water muddy, bottom sand and mud, width 4 to 15 feet, depth to 4 feet, *Chrosomus erythrogaster* dominant.

C5: 38° 57′ 25″ N — 90° 19′ 55″ W; 5-7-67, (150), water muddy, bottom sand and gravel, width 3 to 8 feet, depth to 3 feet, pool and riffles, *Chrosomus erythrogaster* dominant.

Rocky Fork

D1: 38° 56′ 50″ N — 90° 14′ 12″ W; 6-14-67, (190), water turbid and green, bottom sand and rock, width 3 to 20 feet, depth to 3 feet, rock bottom pools and water falls, abundant algae and snails, *Notemigonus crysoleucas* dominant.

D2: 38° 56′ 50″ N — 90° 13′ 15″ W, located on property of Camp Warren Levis, Boy Scouts of America; 7-14-67, (27), water turbid and polluted, bottom sand and mud, width 15 to 20 feet, depth to 3 feet.

D3: 38° 56′ 52″ N — 90° 12′ 50″ W; 6-14-67, (32), water clear, bottom sand, width 3 feet, depth 1 foot, small riffles, *Notropis dorsalis* only species collected.

Little Piasa Creek — East

E1: 38° 59′ 10″ N — 90° 15′ 22″ W; 6-14-67, (401), water clear, bottom sand, width 2 to 10 feet, depth 2 feet, water temperature 31° C, pH 8.5, DH 22°.

E2: 38° 59′ 10″ N — 90° 15′ 22″ W, Little Piasa Creek and Highway 267, 2 miles NW of Godfrey; 4-19-66, (1023), Thomerson and ichthyology class, water clear white, bottom soft sand and gravel, width 5 to 20 feet, depth to 5 feet; 5-26-66, (79), Emons, Grover and Osborne, water turbid, bottom sand and gravel, width to 75 feet, depth to 6 feet, water high; 6-27-67, (1105), water clear, bottom sand, mud, rock and gravel, width 10 feet to 30 feet, depth to 5 feet, current strong over riffles and slow to none in pools.

E3: 38° 59′ 30″ N — 90° 13′ 30″ W; 7-27-67, (65), water clear, bottom sand, mud and rock, width to 12 feet, depth 1 to 2 feet, water temperature 24° C, pH 8.0, DH 18°.

E4: 38° 59′ 50″ N — 90° 12′ 59″ W; 7-6-67, (2148), Smith, Twillman and Thomerson, water clear, bottom sand and gravel, width 5 to 10 feet, depth to 2 feet, some algae present.

E5: 39° 00′ 25″ N — 90° 12′ 30″ W; 7-6-67, (1101), Smith, Twillman and Thomerson, water clear, bottom sand and gravel, width 3 to 8 feet, depth 1.5 feet, some algae present.

E6: 39° 01′ 05″ N — 90° 12′ 05″ W; 7-6-67, (1506), Smith, Twillman and Thomerson, water clear, bottom sand and gravel, width 2 to 4 feet, depth 1 foot, some algae present.

E7: 39° 02′ 35″ N — 90° 10′ 10″ W; 7-21-67, (299), water turbid, bottom rock, gravel and mud, width 3 to 8 feet, depth to 4.5 feet, large rocks on bottom, *Etheostoma spectabile* dominant.

E8: 39° 03′ 10″ N — 90° 09′ 40″ W; 7-21-67, (337), water muddy, bottom sand and mud, width to 3 feet, depth to 1 foot, very small.

Little Piasa Creek — West

F1: 39° 00′ 55″ N — 90° 16′ 10″ W; 6-20-67, (519), water slightly turbid, bottom mud and gravel, width 5 to 15 feet, depth 2 feet.

F2: 39° 01′ 35″ N — 90° 17′ 29″ W; 6-20-67, (722), water slightly turbid, bottom mud and rock, width 5 to 10 feet, depth 2.5 feet.

F3: 39° 02′ 22″ N — 90° 17′ 53″ W; 6-20-67, (1158), water slightly turbid, bottom sand and rock, width 1 to 5 feet, depth 2 feet, some algae present.

F4: 39° 02′ 36″ N — 90° 15′ 55″ W; 6-20-67, (427), water slightly turbid, bottom mud and gravel, width 15 feet, depth 3.5 feet, large pool, several large *Ictalurus melas* taken.

Minor Species

A total of 44 species were taken from the drainage during this study and undoubtedly other species occur in the lower portion of Piasa Creek. Due to heavy public usage and other factors we were not able to adequately sample the creek below site B1. The total number of each species collected is followed by sites and respective numbers in each collection. Where a site was collected more than once the numbers of specimens in each collection are listed in chronological order as in the Site Accounts. Nineteen species taken only in the lower portions of the drainage are as follows: *Dorosoma cepedianum* (LeSeuer), gizzard shad; Total—65, A1—13; B1—9, 38; B3—1, 5. *Hiodon alosoides* (Rafinesque), goldeye; Total—1; B1—1. *Carpiodes carpio* (Rafinesque), river carpsucker; Total—16; B1—4, 10, 2. *Carpiodes cyprinus* (LeSeuer), quillback; Total—113; B1—1, 18; B3—4, 27, 55; B4—2; B5—4; B6—2. *Ictiobus bubalus* (Rafinesque), small mouth buffalo; Total—17; B1—10, 6; B3—1. *Ictiobus cyprinellus* (Valenciennes), bigmouth buffalo; Total—1; B1—1. *Cyprinus carpio* Linnaeus, carp; Total—2; B1—1; C1—1. *Notropis blennius* (Girard), river shiner; Total—66; B1—58, 8. *Pimephales vigilax* (Girard), bullhead minnow; Total—5; B1—5. *Ictalurus punctatus* (Rafinesque), channel catfish; Total—17; B1—11, 6. *Labidesthes sicculus* (Cope), brook silverside; Total—7; B1—2, 5. *Aphredoderus sayanus* (Gilliams), pirate perch; Total—1; B1—1. *Morone crysops* (Rafinesque), white bass; Total—10; B1—1, 9. *Morone mississippiensis* (Jordan and Eigenmann), yellow bass; Total—3; A1—3. *Pomoxis annularis* (Rafinesque), white crappie; Total—6; A1—6. *Pomoxis nigromaculatus* (LeSeuer), black crappie; Total—2; A1—1; B1—1. *Percina caprodes* (Rafinesque), logperch; Total—4; B1—4. *Stizostedion vitreum* (Mitchill), walleye; Total—9; B1—9. *Aplodinotus grunniens* (Rafinesque), freshwater drum; Total—7; A1—1; B1—2, 4.

Dorosoma cepedianum was taken from pools in muddy water. With the exception of one large specimen, 12 in. long (Total Length), all specimens were under 6 in. long. Of the two species of carpsucker found *C. cyprinus* was the most numerous. Larger specimens (to 9.5 in.) were taken at B1 but all specimens taken upstream from there were young of the year. The *C. carpio* taken only at B1 were all small, less than 6 in. long. All buffalo were small (to 3 in.). Carp are probably common in the lower portion of the creek but only two small specimens were taken. *Notropis blennius* was found in fair numbers at B1 but probably does not ascend much further upstream. *Ictalurus punctatus* were taken only at B1 and all were small (to 5 in.). *Morone crysops* were more common than *Morone mississippiensis* but all specimens of both species were small, less than six in. long. The single collection of *Percina caprodes* was made from a deep pool at B1 when the river was high and flow at this site was reversed. *Stizostedion vitreum* taken at the same time were under seven in. long. All *Aplodinotus grunniens* were six in. or less.

Seven other species were taken only in small numbers. They are as follows: *Moxostoma erythrurum* (Rafinesque), golden redhorse; Total—34; B1—1, 1, 7, 12, 5; B3—3, 3; E2—2. *Hybognathus nuchalis* Agassiz, silvery minnow; Total —14; B1—5, 6; B3—2; B4—1. *Notemigonus crysoleucas* (Mitchill), golden shiner; Total—130; A1—3; B1—3; B4—1; C4—1; D1—115; D2—1; E2—6. *Phenocobius mirabilus* (Girard), suckermouth minnow; Total—12; B1—3; B3—2, 1, 1; B7—1; B8—1; C3—1; E2—2. *Gambusia affinis* (Baird and Girard), mosquitofish; Total—1; B1—1. *Lepomis humilis* (Girard), orangespotted sunfish: Total—26; A1—14; B1—2, 1, 1, 2; B3—1, 3; B4—1; E2—1. *Micropterus salmoides* (Lacépède), largemouth bass; Total—7; B1—1; B3—1, 1; B4—2; C1—1; E2—1.

Except for a single specimen 15 inches long all *M. erythrurum* were under eight inches long. Trautman (1957) stated that the golden redhorse "was intolerant of continuous turbidity and rapid silting of stream bottoms." This may be the reason for its uncommon occurrence in Piasa Creek. *Hybognathus nuchalis* were collected only from large pools in the main creek and are probably more abundant in the lower portion of Piasa Creek than our sample indicates. *Notemigonus crysoleucas* were rare with exception of site D1 where they are dominant. Apparently the reduction of other competing species in this area as a result of pollution has allowed the golden shiner to establish a sizable poulation.

As is usual when several riffle dwelling minnows are present, *P. mirabilus* is rare but was found at scattered localities throughout the drainage. Though only a single *G. affinis* was taken, several dozen were seen but not collected at D3 on 21 September 1967. *Lepomis humilis* was found in good numbers around the shore at the mouth and is probably common in the lower reaches of Piasa Creek. The few specimens of *M. salmoides* collected were all young of the year. In the course of collecting fishes for this study we also took a number of crayfish belonging to the genus *Orconectes*. The common crayfish in Piasa Creek is *O. virilis*. Less common is *O. immunis*.

Major Species

The relative abundance of these common species at all collecting sites is given in Table 1.

Catostomus commersoni (Lacépède), white sucker; Total—528; B1—3, 18; B3—118, 41, 78; B6—4; B7—30; B8—14; C1—1; C4—3, 81; C5—1; E1—2; E2—11, 28; F1—29; F2—45; F3—10; G1—11. This is the common sucker in the drainage and made up 2.2% of the total sample. White suckers were found in all of the larger portions of the drainage system. Males running milt were taken on 19 April 1966 and young of the year on 14 June 1967. Large specimens were taken from all of the deep pools sampled in the main creek. At E2, in addition to the 28 specimens retained, 13 large individuals were released. We would agree with Trautman (1957) that white suckers "appeared to be more tolerant to turbidity, siltation and other organic and inorganic pollutants than any other sucker." The white sucker was found in all branches of the drainage with the exception of Rocky Fork. Apparently this branch is too polluted for even this tolerant species.

Campostoma anomalum (Rafinesque), stoneroller; Total—4436; B2—4; B3—163, 217, 401; B4—48; B5—53; B6—70; B7—58; B8—83; C1—3; C2—61; C3—1; C4—31, 470; C5—22; E1—85; E2—221, 40, 60; E3—12; E4—84; E5—195; E6—360; E7—77; E8—163; F1—112; F2—171; F3—870; F4—178; G1—123. The stoneroller is a common species in the

drainage, 18.8% of the total sample. Outside of Rocky Fork, it was found at all sites except three. Although there seems to be much suitable habitat and food it is presumably excluded from Rocky Fork by pollution. Young of the year were first taken in June.

Chrosomus erythrogaster (Rafinesque), southern redbelly dace; Total—430; C3—2; C4—68, 295; C5—65. The southern redbelly dace occurs as an isolated population in upper Mill Creek, and was taken at only three localities. In collections from C4 and C5 it comprised 25.5% to 43.3% of the sample. Forbes and Richardson (1908) did not report *Chrosomus erythrogaster* from this part of the state. The presently known state distribution of the species, however, includes the northeastern quarter of the state and 2 small areas in the central part of the state, one of which includes the study area. A single collection has been made in Jackson County in southern Illinois (Phillip W. Smith, personal communication).

Notropis atherinoides (Rafinesque), emerald shiner; Total—869; A1—4; B1—118, 10, 197, 430, 5; C1—96, 9. The emerald shiner was collected only in the lower portions of the drainage. This agrees with Smith (1965) who lists it as occurring in the larger and medium sized rivers throughout Illinois. It is abundant where found and may make up as much as 55% of the sample.

Notropis dorsalis (Agassiz), bigmouth shiner; Total—8032; B1—84, 38, 1, 4, 5; B2—103; B3—1144, 559, 659; B4—71; B5—141; B6—462; B7—156; B8—226; C1—4; C2—6; C3—17; C4—27, 18; C5—20; D1—7; D3—32; E1—249; E2—65, 300; E4—1689; E5—607; E6—591; E7—47; E8—35; F1—119; F2—232; F3—102; F4—28; G1—183. The bigmouth shiner made up 34.2% of the total sample and is the dominant species throughout the drainage. It was collected at all but five sites and in most collections makes up from 25% to 78% of the sample. Young of the year first appeared in collections on 20 June.

Notropis lutrensis (Baird and Girard), red shiner; Total—1093; B1—38, 17, 124, 12, 4; B3—93, 29, 168; B4—21; B6—1; B7—2; B8—6; C1—79, 53; C2—2; C3—11; C4—9, 3; C5—1; D1—13; E2—66, 13, 200; F1—2; F2—18; F4—96. The red shiner was found generally throughout the system but only amounted to 4.6% of the total sample. It usually made up about 10% of the sample when present. Forbes and Richardson (1908) reported that *Notropis lutrensis* spawns from the middle of May to the last of June. However, we collected young of the year as early as April and males in breeding color were taken as late as October 29.

Notropis stramineus (Cope), sand shiner; Total—603; B1—41, 40, 17; B2—11; B3—96, 89, 40; B4—30; B5—8; B6—5; B7—3; B8—2; C1—8; C3—11; C4—11, 41; E1—21; E2—42, 67; F1—4; G1—16. The sand shiner was found in the deeper portions of the drainage mainly over sand bottom. Young of the year were collected as early as April. It was not found as far upstream as *N. dorsalis*.

Notropis umbratilus (Girard), redfin shiner; Total—215; B1—1; B3—10, 7, 15; B4—8; B7—3; C1—8; C2—5; C3—31; C4—20, 3; D1—1; E2—49, 54. The redfin shiner was found throughout the system, but seldom in large numbers. Forbes and Richardson (1908) reported females bursting with eggs were taken about the first of June. This agrees with our taking the first specimens of young of the year on 27 July.

Pimephales notatus (Rafinesque), bluntnose minnow; Total—971; B1—7, 12, 23, 5; B2—2; B3—29, 8, 60; B4—9; B7—2; C1—3, 66; C2—4; C3—30; C4—34, 41; C5—13; D1—6; E2—399, 180; F1—11; F2—7; F3—3; F4—17. The bluntnose minnow was found generally distributed throughout the drainage and makes up 4.1% of the total sample. Males and females in breeding condition were first collected on 7 May. Forbes and Richardson (1908) reported the spawning season in central Illinois as May 15 to June 15. This agrees with our data.

Pimephales promelas (Rafinesque), fathead minnow; Total—167; B1—2, 1; B3—13, 5, 5; B4—9; B9—56; C1—1; C4—1; D1—25; E2—3, 13; F1—5; F2—26; F3—2; F4—3. The fathead minnow was found throughout the drainage over mud bottom. Males in breeding condition were taken on 26 May. Trautman (1957) stated "the fathead and bluntnose minnows were competitors, and the fathead occurred in greatest population densities only where the bluntnose was absent or comparatively few in numbers." The fathead was abundant at only two sites, D1 and B9. At these sites it made up 13.1% and 44.0% of the total sample respectively. The bluntnose on the other hand made up only 3.1% of the sample at D1 and was absent at B9. At all sites where the bluntnose was abundant the

fathead did not exceed 1.5% of the total sample.

Semotilus atromaculatus (Mitchill), creek chub; Total—3071; B1—36; B2—7; B3—54, 95, 278; B4—51; B5—45; B6—278; B7—83; B8—175; C1—6; C2—30; C3—6; C4—12, 58; C5—16; D1—5; D2—23; E1—16; E2—42, 104, 16; E3—53; E4—105; E5—156; E6—391; E7—67; E8—129; F1—123; F2—209; F3—104; F4—62; G1—200. The creek chub occurred at all but three sites and was the third most abundant species (13.1% of the total sample). Ripe females were taken on 19 April and the first young of the year were taken on 14 June.

Ictalurus melas (Rafinesque), black bullhead; Total—53; B1—1, 1, 1; B3—1; B9—1; C1—18; E2—5, 5; E4—1; E7—6; E8—1; F4—11. The black bullhead was taken mainly from the deep mud-bottomed pools throughout the drainage.

Ictalurus natalis (LeSeuer), yellow bullhead; Total—21; B3—1, 7, 4; B4—1; B8—1; C1—3; C3—2; C4—1; C5—1. The yellow bullhead was found in only the main branch of Piasa and in Mill Creek in larger pools. Forbes and Richardson (1908) reported that the black and yellow bullheads show an observable tendency of local separation. Our data tend to support this statement.

Fundulus notatus (Rafinesque), blackstripe topminnow; Total—166; B1—7, 12, 94; B2—5; B3—1, 1, 26; C1—6, 4; C3—2; C4—3; E2—4, 1. The blackstripe topminnow was taken from sites which contained relatively still water in large pools with an abundance of debris present. An extremely variable sized population exists downstream from B1, at times quite large.

Lepomis macrochirus Rafinesque, bluegill; Total—239; A1—16; B1—3, 7, 32, 18, 1; B2—2; B3—9, 9, 9; B4—20; C1—3, 7; C2—18; C3—2; D1—14; D2—3; E2—5, 1, 4; E4—1; E7—9; G1—1. The bluegill is scattered throughout the drainage in small numbers.

Lepomis cyanellus Rafinesque, green sunfish; Total—281; A1—1; B1—1, 4, 3, 6; B3—3, 6, 11; B4—2; B7—5; B8—2; B9—70; C1—7; C3—5; C4—14, 13; C5—4; D1—4; E2—39, 2; E4—7; E5—4; E6—26; E7—7; F1—2; F2—1; F4—26; G1—6. The green sunfish is ubiquitous throughout the system.

Etheostoma spectabile (Agassiz), orangethroat darter; Total—1681; B1—7, 2; B2—9; B3—52, 80, 36; B4—7; B5—27; B6—90; B7—68; B8—164; C2—1; C3—1; C4—11, 94; C5—5; E1—28; E2—103, 3, 5; E4—261; E5—139; E6—138; E7—86; E8—9; F1—112; F2—13; F3—31; F4—6; G1—93. The orangethroat was found in abundance throughout the drainage, with the exception of Rocky Fork. It was collected mainly from the riffles although many specimens were taken from shallow pools. The orangethroat was the fourth most common species and made up 7.1% of the total sample.

Cottus carolinae (Gill), banded sculpin; Total—43; C4—7, 34; C5—2. The banded sculpin was found at only two localities and in association with the redbelly dace. During the day it was kicked from the riffles but at night it was taken from all parts of the stream. Forbes and Richardson (1908) reported a single collection of the banded sculpin from a spring in Jersey County. The previously known statewide distribution included a small area just north of Piasa Creek in west central Illinois and the extreme southern tip of the state (Phillip Smith, personal communication).

Discussion

The lower portion of Piasa Creek at least upstream to B1 is a highly unstable habitat under the influence of the river and has certainly been greatly altered by the operation (since 1938) of the Lock and Dam No. 26 on the Mississippi River below the mouth of the creek at Alton, Illinois. When the river level is high, turbid water flows upstream at least as far as B1 and C1 (see Site Accounts) and considerable silt and mud are deposited on the bottom. When the river level is low and the creek flow high, there is scouring of the silt to leave a clean sand and gravel bottom under clear flowing water. There seems to be considerable movement of fishes into and out of this area (around B1) depending on water and bottom conditions. A total of 38 out of the 44 species collected in the system were taken at B1 but no more than 26 in a given

collection. Below B1 many more river species probably occur than our data would indicate. Below this point the creek becomes too large for effective collecting, the banks are private property and there is considerable boat traffic.

The *Chrosomus erythrogaster* record from Mill Creek is of considerable interest. The nearest Illinois records are from northeastern Calhoun and southeastern Pike counties, west of the Illinois River (Phillip W. Smith, personal communication). Though *C. erythrogaster* occurs north of the Missouri River on the Missouri side of the Mississippi these populations are separated from Piasa Creek by broad areas of unsuitable habitat. The *Cottus carolinae* record is of similar interest, however, this species has been taken in Otter Creek, a Macoupin Creek tributary just northwest of Mill Creek (Lopinot, 1968). No *C. erythrogaster* were taken at the Otter Creek locality and large numbers of *N. lutrensis* were present (34.4% of total sample, Lopinot, 1968, p. 114, Table 4). At the sites in upper Mill Creek *N. lutrensis* is present but not abundant. We suspect that *C. erythrogaster* and *C. carolinae* may have been present both in Rocky Fork and the lower portion of the main creek before these areas were altered by human activity. Before the lower portion of the creek was flooded, it was probably a clean gravel and sand bottomed stream. Rocky Fork was probably a clear flowing rock and gravel bottomed stream before the advent of pollution from several sewage lagoons draining into the creek above Warren Levis Lake as well as seepage from septic tanks serving the many subdivisions in the area. A large fish kill, with largemouth bass as large as 4 or 5 pounds dying, was observed by Smith in the spring of 1965. The kill occurred in Warren Levis Lake and in Rocky Fork downstream from the lake.

Other than the species discussed above, the fishes of Piasa Creek include wide ranging and ubiquitous Upper Mississippi Valley small stream fishes such as *Notropis dorsalis*, *N. stramineus*, *N. lutrensis*, *N. umbratilis*, *Semotilus atromaculatus*, *Pimephales notatus*, *Campostoma anomalum*, *Catostomus commersoni*, *Lepomis cyanellus*, and *Etheostoma spectabile*.

Acknowledgments

We wish to thank the various class members and others, as listed with the Site Accounts, for their assistance in making collections. Mr. William L. Pflieger, Missouri Department of Conservation, and Dr. Phillip W. Smith, Illinois Natural History Survey, aided with identification of fishes. We also wish to thank Dr. Smith for his courtesy during our visits to his institution. Dr. Dorothy Gore, Southern Illinois University at Edwardsville, supplied the information on the geology of the region. Crayfish identifications were made by Mr. Rollin D. Reimer, Oklahoma State University.

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EVALUATION OF THE CONTRIBUTION OF FORAGE MATERIAL TO THE *IN VITRO* CULTURE OF ISOTRICHA

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ABSTRACT. — In order to define more clearly the contribution of forage materials to the *in vitro* culture of Isotricha several concentrations of dehydrated alfalfa meal were compared with similar concentrations of corn seedlings of unique chlorophyll status: normal, albino mutant, and etiolated. The ciliates were obtained from strained rumen fluid by gravitational procedures after incubation with fructose (3% w/v). A modified culture medium with no rumen fluid maintained Isotricha populations for as long as 30 days. A comparison of 0, 10, 30, and 50 mg of the alfalfa meal per day showed that population numbers and days of culture life were proportional to the amount of alfalfa provided. Mutant (one gene A/a), albino and etiolated corn seedling significantly depressed ciliate cultures as compared with alfalfa meal, which was significantly superior to normal corn seedling in maintaining these populations. The growth factors appear to be associated with chlorophyll or photosynthetic products.

The role of ciliate populations in the rumen has been difficult to evaluate because of the failure to obtain an axenic culture or to obtain growth in chemically defined culture media, as for example, has been possible in the case of Tetrahymena, Kidder (1951) with one exception, Samoya (1953). Those extended *in vitro* cultures of rumen ciliates reported to date contained chlorophyll material or rumen fluid in the medium, as well as rumen bacteria. Insofar as the diet of the rumen fluid donor animal includes green plant material it can be assumed that the rumen fluid is also a medium rich in these constituents. It is evident that there is further need for clarification of the growth factors which are contributed by these constituents. It was, therefore, the objective of this investigation to further define the nature of the chlorophyllus plant material required for the *in vitro* culture of the holotrich genus, Isotricha. These ciliates of the order Trichostomatida have been cultured previously for limited periods of time; Sugden and Oxford (1952), Gutierrez (1958), Quinn *et al.* (1962), Purser and Weiser (1965), and Clark and Hungate (1966).

MATERIALS AND METHODS

Nearly pure populations of Isotricha were obtained by employing a procedure similar to that of Gutierrez (1958). Strained rumen fluid was diluted (1:1) with salt buffer solution and incubated at 40° C. with D-fructose (3% w/v) for 30 to 60 minutes in a separatory funnel. The resulting amylopectin-laden Isotricha possessed a higher specific gravity than the Entodiniomorphida. Conse-

TABLE 1.—*Isotricha* Culture Medium

Ingredients	Concentration/liter
K_2HPO_4	1.0 grams
NACl	3.0 grams
$MgSO_4.7H_2O$	0.1 grams
$CaCl_2$	0.1 grams
$NaHCO_3$	5.0 grams
Streptomycin sulfate	20.0 mg
$Na_2S.9H_2O$	1.0 gram
Resazurin	0.01 grams
Starch (corn & wheat)	0.6 grams
Gas	100% CO_2

quently, they could be separated gravimetrically in the separatory funnel and then by layering on a column about 1 M long containing warmed (40° C.), gassed (CO_2) salt solution. Dasytricha could be lysed by layering 8 cm. of distilled water over the salt solution.

The culture medium (Table 1) was derived mainly from Mah (1964) and Rahman *et al.* (1965). The $NaHCO_3$ solution was autoclaved separately from the other ingredients and mixed after cooling. The medium was warmed (40° C.), gassed (CO_2) and 25 ml were carefully pipetted into 40 ml round-bottom centrifuge tubes. The ciliates had been appropriately diluted with the warmed and gassed salt solution. Cultures were mixed well to assure uniform distribution and were added to the medium in 2 ml aliquots. Bacteria were introduced in 2 ml aliquots of fresh rumen fluid, centrifuged on the initial culture day to eliminate protozoa. Starch and streptomycin were added, the culture tubes were gassed with CO_2, 0.05% $NaS{\cdot}9H_2O$ was added, and the tubes were stoppered.

Forage Material

Alfalfa meal was pulverized in a Wiley mill and stored in a sealed container in the dark. The mutant corn (GC-100 Carolina Biological Supply Company) was a single gene A/a chlorophyll mutant (3:1). The plants were cultivated in washed sand in a growth chamber, and harvested two weeks after planting. The etiolated corn was obtained by growing normal dent corn in a darkened room for a similar period of time. The seedlings were harvested, air dried. pulverized in a Wiley mill, and stored in a sealed flask in the dark. These plants differed in that the mutant had no chlorophyll and no protochloroplast whereas the etiolated had slightly modified chlorophyll (—2H) and contained a protochloroplast. Both had the carotenoid pigments.

Enumeration:

Counts of Isotricha were made by placing 1 ml of an apropriate dilution of the culture in a petri dish, in an area ringed with vaseline to contain the medium. The population was counted by placing the dish over a darkly lined grid. The ciliates were observed for motility and stained with Lugol's Iodine, facilitating their identification with a dissecting microscope (X 20).

Culture Maintenance

Cultures were maintained for longer periods by centrifugation at low speeds to concentrate the ciliates and then 12.5 ml of the supernatant was removed. Cultures could also be maintained by removing the top one-half of the undisturbed culture daily or on alternate days. The majority of ciliates remained near the bottom of the culture vessel. Twelve and one-half ml of fresh, warmed gassed medium was added to each tube, which was, in turn, gassed with CO_2 that had additional $NaS{\cdot}9H_2O$. The tubes were then stoppered.

In the short-term comparisons of forage a similar procedure was followed except that the culture was thoroughly mixed by shaking and then one-half of it was removed and replaced by fresh medium.

Results and Discussion

The initial experiments were designed to evaluate the rumen ciliate culture procedures attributed to Coleman (1960), Purser and Weiser (1965), Rahman *et al.* (1964), Hungate (1950) and Mah (1964). The number of ciliates and the longevity in days were used as criteria of success. Rumen fluid was essential only on the initial culture day probably as a bacterial inoculant. Subsequent additions produced more variability than if no rumen fluid was provided. Use of rumen fluid seemed undesir-

able also in view of its chemically ill defined nature.

A suction method was superior to centrifugation because less time was required, less aeration and cooling occurred, and the possibility of trapping ciliates in pellet debris was eliminated. I s o t r i c h populations could be maintained *in vitro* for several weeks. The longest continuous culture was for 30 days and perhaps could have been extended further but was terminated because of other obligations. Population levels gradually declined suggesting that not all the requirements for the extended *in vitro* culture of this organism were met. It may also be a consequence, however, of an insufficiently rapid *in vitro* reproduction r a t e which has been tabbed at 48 hours by Gutierrez (1958), in which ciliates were lost by medium replacement. The 48 hour rate of reproduction does not appear to be realistic in view of rumen passage data rate. It is, however, conceivable that this situation can be modified by a chemotaxis stimuli which attracts the Isotricha to ingesta distal to the sulcus omassi.

A randomized complete block experimental design was employed to evaluate the effect of chlorophyll on the cultures (Table 2). Four forage treatments consisted of the chlorophyll mutant, etiolated and normal corn seedlings, and dehydrated alfalfa. Each treatment was provided at three levels (10, 30 and 50 mg per culture) and each level had four replications, in each of which the *Isotricha* were counted twice and the mean values recorded.

Insofar as numbers of *Isotricha* were fairly constant on the first three culture days, these counts were not subjected to statistical analysis. Each successive day's treatment was performed separately in view of the substantial day-to-day population change. This was due, in part, to the culture dilution technique employed.

No significant differences could be demonstrated between any of the corn treatments on the fourth day, ($P>0.05$). Alfalfa, however, supported larger numbers than the other treatments ($P<0.05$) on the fourth day. On the fifth day no differences were evident between the mutant and etiolated corn. On each succeeding day, however, the etiolated corn was significantly s u p e r i o r, ($P<0.05$). Similarly, normal corn and alfalfa were significantly superior to the non-chlorophyllous treatments on each following culture day, but not from each other on the fifth or sixth day. The alfalfa was clearly superior to the normal c o r n on the seventh and eighth day. A higher level of sugar in alfalfa than in corn may be responsible. The protein was higher (26% CP) in the corn than in the alfalfa (18% CP). It was also apparent that the 50 mg levels of the alfalfa and normal corn were more effective than other treatments in maintaining *Isotricha* populations, whereas 50 mg levels of mutant and etiolated corn were significantly superior to all lower levels of mutant corn and after day five were not different from 30 mg levels of normal corn. Similarly, 10 and 30-mg levels of etiolated corn did not differ from similar levels of normal corn except on day eight, ($P<0.05$).

These data strongly suggested that chlorophyll, or perhaps some aspect of the porphyrin ring structure, is the first limiting nutrient in this *in*

TABLE 2.—Comparison of Four Forages at Three Levels 10, 30, & 50 mg per 40 ml Culture per day. Data Expressed as Isotrichs/ml

Block	Yellow			Etiolated			Green			Alfalfa			Day
	10mg	30mg	50mg	10mg	30mg	50mg	10	30	50	10	30	50	
A.....	400	188	264	208	249	255	212	198	210	172	390	476	
B.....	360	310	360	250	284	254	338	516	555	326	611	657	4
C.....	348	259	257	272	292	279	295	356	519	410	415	535	
Total.	1,108	757	881	730	825	788	845	1,070	1,284	908	1,416	1,668	
A.....	32	16	10	89	124	45	36	70	80	60	110	147	
B.....	251	169	15	116	133	36	256	283	316	163	192	383	5
C.....	160	87	19	97	117	33	123	188	208	131	138	159	
Total.	443	272	44	302	374	114	415	541	604	354	440	689	
A.....	0	1.0	0	36	24	3.0	4	16	20	4	35	96	
B.....	69	42	0	43	58	1.0	72	84	100	74	74	110	6
C.....	35	9	0	27	58	4.0	75	79	97	36	57	84	
Total.	104	52	0	106	140	8.0	151	179	217	114	166	290	
A.....	0	0	0	5	6	0	0	0	8	1	11	68	
B.....	4	5	0	8	13	0	14	16	22	28	50	66	7
C.....	4	0	0	9	11	0	7	13	14	15	29	40	
Total.	8	5	0	22	30	0	21	29	44	44	90	174	
A.....	0	0	0	0	1.0	0	0	0	0	0	4	11	
B.....	0	0	0	0	0.0	0	2	3	4	8	16	23	8
C.....	0	0	0	0	2.0	0	0	2	4	3	15	28	
Total.	0	0	0	0	3.0	0	2	5	8	11	35	62	

vitro Isotricha culture. The lack of stimulation effect of the etiolated corn at the 50-mg level indicated that some aspect of the complete chloroplast or a product of photosynthesis is also required. Further study regarding lipid or tocopherol requirements is, therefore, indicated. These data were somewhat confounded in that it is impossible to distinguish between bacterial and ciliate requirements. Bacteria, however, can be cultured without a chlorophyllous constituent, Caldwell and Bryant (1966) although *Bacteroides* ruminicola requires hemin Caldwell *et al.* (1966).

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EVAPOTRANSPIRATION CONTRASTS BETWEEN SOUTH-FACING BLUFF PRAIRIES AND NORTH-FACING FOREST

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ABSTRACT. — A short term study was set up to test the suitability of the evaporimeter and transpirometer for the measurement of ecological differences as well as their ability to measure evaporation and transpiration differences quantitatively under three habitats. The correlation between the evaporimeters and transpirometers indicate that one might be able to predict transpiration rates from evaporimeters with sufficient data. However certain difficulties were encountered with the two systems of measurement.

Along the exposed south and southwest facing bluff slopes of the Mississippi, small bluff or hill prairies occur frequently, despite the dominance of oak-hickory forest throughout this river-border area (Vestal, 1931). The southerly slopes, being hotter and drier, support grassland and open forest; while on the northerly slopes dense forest prevails.

The floristics of these xeric grasslands, usually dominated by little bluestem (*Andropogon scoparius* Michx.) have been detailed by Evers (1955), while the vegetation-soil relationships have been discussed by Kilburn and Warren (1963) and Bland and Kilburn (1966).

Microclimatic studies at Principia College, Jersey County, Illinois, indicate far different climatic regimes in several aspects between these exposed sites and the more protected forest habitat: higher soil maxima, higher air maxima in all months, lower relative humidity and far stronger wind velocities. All of these features result in increased transpiration rates, probably the primary factor in the encouragement of grasses and discouragement of trees.

In order to assess quantitatively evapotranspiration differences between these habitats two inexpensive, yet readily comparable techniques for the measurement of evaporation and transpiration were selected. These have been used successfully by Davenport (1965) in England. The techniques include "evaporimeters," shallow glass dishes measured daily for water loss, and "transpirometers," small sealed flowerpots of grass. A short term study was set up at Principia College to test the suitability of the evaporimeter and transpirometer for the measurement of ecological differences and to test the ability of these techniques to measure quantitative differences in evaporation and transpiration in three differ-

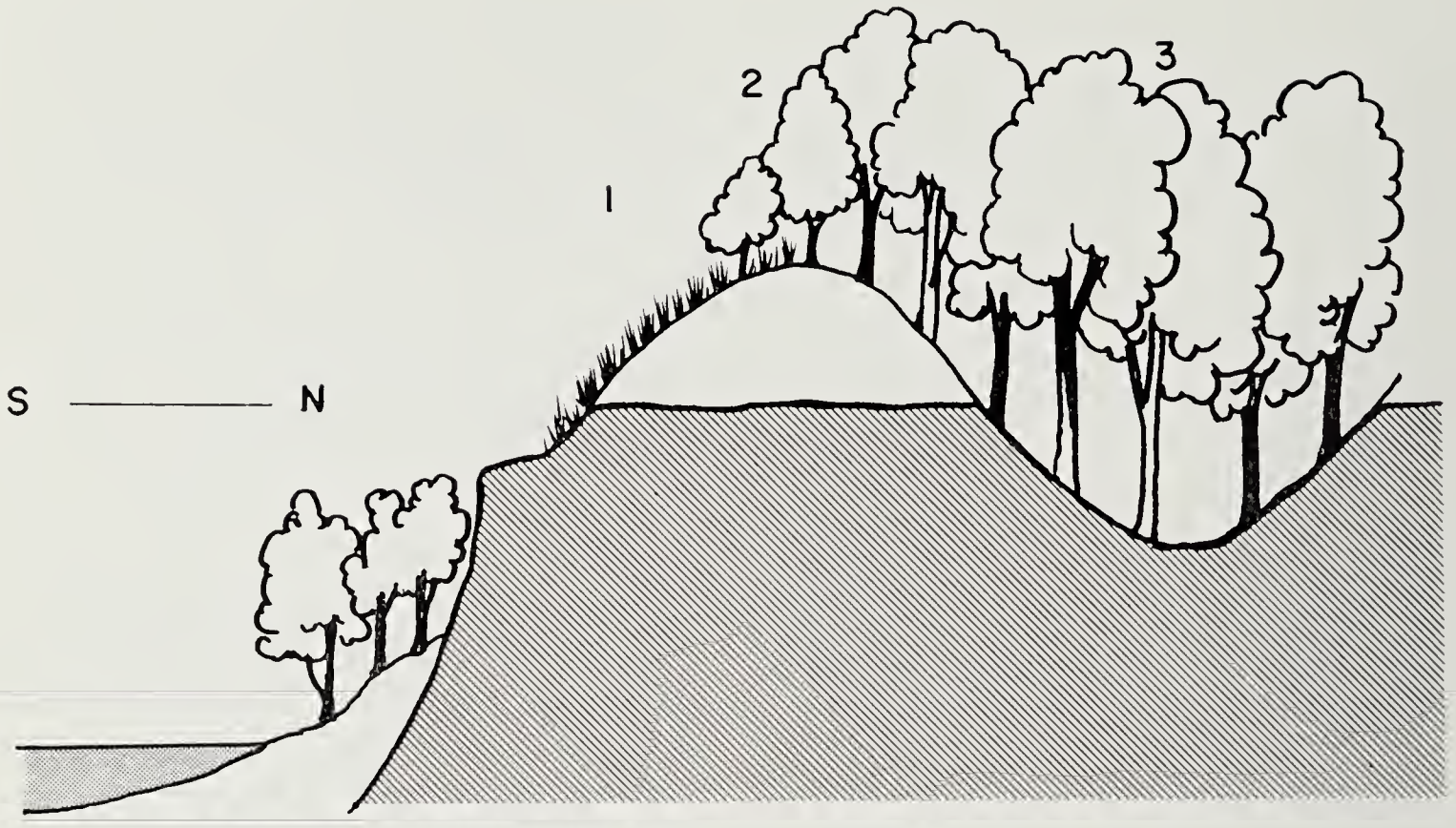

FIGURE 1. — Location of Stations. The prairie station (1) faces south overlooking the Mississippi River; the ecotone station (2) is at the crest of the bluff; and station (3) is in the north-facing ravine forest. The shaded subsoil is limestone bedrock; the upper unshaded portion above the limestone is loess.

ent habitats. This paper reports the results of this study.

MATERIALS AND METHODS

Three field stations were set up for this study and they were designated 1, 2, and 3 on Figure 1. Station 1 was on the bluff prairie, station 2 was located on the crest (ecotone), and station 3 was located in the deep ravine immediately adjacent, in Sec. 21, T. 6N., R. 11W. of Jersey County.

Each station was set up with nine transpirometers and two evaporimeters. Three transpirometers were at ground level while the remaining transpirometers and the evaporimeters were placed in iron rings which were attached to a metal post standing six feet above ground level. An evaporimeter was placed at the three and five foot levels while two transpirometers were placed at the two, four and six foot levels.

Temperatures were recorded by a Taylor maximum-minimum thermometer checked daily at 5:30 p.m. Glass dishes 10 cm. in diameter and 4 cm. in depth were used as evaporimeters. These glass dishes were placed on platforms painted black in the center and white on the outside, the scheme used by the more streamlined English models. At the beginning of each 24 hour measuring period, the evaporimeters were filled with 200 ml. of water. The amount of water lost over the 24 hour period was considered the amount evaporated.

Grass transpirometers were patterned closely after those used by Davenport (1965). The drainage holes of plastic pots (12.5 cm. in diameter x 12 cm. deep) were plugged and the pots were then filled to within 2 cm. of the rim with soil. The soil was wetted down and sown with 1.5 g. of commercial red top grass seed (*Agrostis* sp.). Germination began six days after sowing and fourteen days later the seedlings were clipped for the first time level with the rims of the pots. They were clipped to the same height every other day thereafter. Field capacity for these pots was established by noting the weight of an unplugged pot containing saturated soil that no longer drained. At the beginning of each 24 hour measuring period each transpirometer was brought up to a field capacity of 1300 grams by adding water. The dense cover of the grass in the transpirometer reduced soil evaporation to a negligible amount so that weight losses were assumed to result entirely from transpiration, a conclusion reached also by Davenport (1965).

TABLE 1.—Maximum temperature differences (means) between north and south slopes. In each month south slope values were higher.

Month	Mean Values, F°
January-February	8.1
March-April	3.7
May-June	19.9
July	13.1
August	18.2
September	20.2
October	8.1
November-December	6.5
Average, May through September	17.8

RESULTS

The maximum temperatures shown in Table 1 reveal a striking contrast between the higher maxima of the exposed bluff region and the protected woody ravine in every month. These differences, with a mean exceeding 20 degrees (F) in September were recorded in an intensive study in 1960-61, and are known to be typical from frequent intermittent measurements since that time. Minimum temperatures from the two habitats, in contrast, show far greater similarity and no consistent pattern. All temperatures were recorded in the shade 1″ above ground level. It is clear that the exposed bluff habitat is not simply warmer; rather it exhibits a far more extreme climatic variation.

Figure 2 (B and C) illustrates the way in which transpiration and evaporation rates from the transpirometers and evaporimeters correspond with the maximum temperature changes. The correspondence of the peaks on the 15th of April is indeed striking, while those on the 28th are also very similar. At the same time, the readings from station 2 (ecotone) and station 3 (north-facing forest) are consistently lower than station 1. In part this reflects the lower temperatures there, but it also reflects the lower wind velocity and higher humidity found there. Recent readings made since the study indicate that wind velocities at station 3 are on the average about 1/10 that of station 1; humidities are consistently lower as well as station 1.

Quantitative comparisons of evaporation can be made from Table 2. Here it can be seen that evaporation from the evaporimeters at station 1 was about 4 times as great in hot days of April and May as station 3, slightly less than this on cooler days, and perhaps even greater than this on cool days in May, after foliage had come out fully on the trees. Data from the transpirometers do not reveal quite such extreme differences. On the hottest days transpiration was nearly three times as great at station 1 as at station 3. Differences between pots at different heights were not consistent and are not shown. This is undoubtedly because of both the variability in the transpirometers, and the small number of them used in the study.

DISCUSSION

It is well known (cf. Meyer and Anderson, 1952) that evapotranspiration is correlated with air temperatures. Therefore, the close correlation between the maximum temperature curves and losses from both the evaporimeters and transpirometers indicates their potential value as tools in comparative ecology. At the same time it is clear that the rates of both are affected by humidity and wind

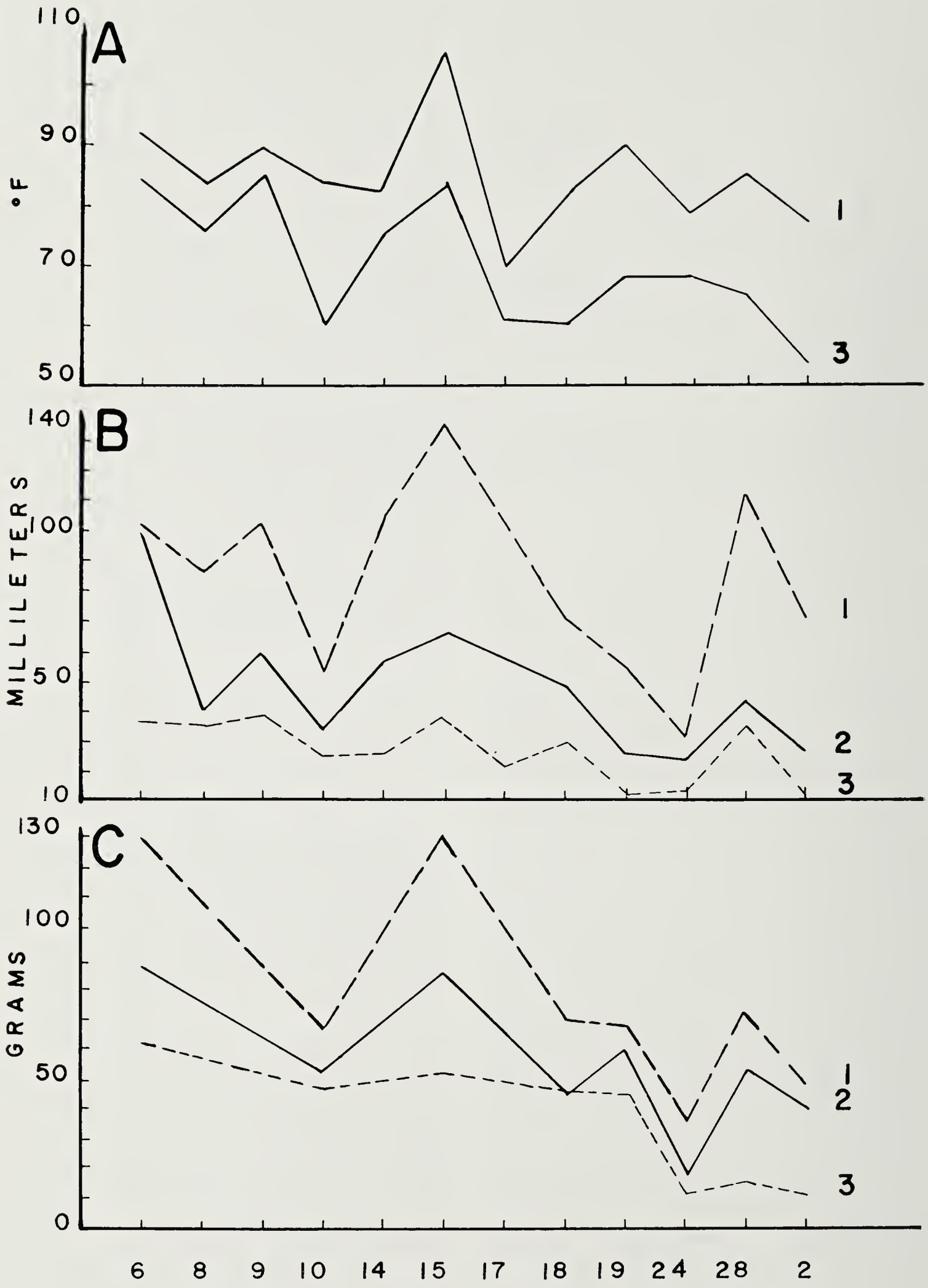

FIGURE 2. — Comparisons of maximum temperatures (A), evaporimeter losses in milliliters (B), and transpirometer losses in grams (C). Readings represent results during April-May 1967. Line 1 based on readings from station 1 in the south-facing hill prairie; line 2 is from station 2; line 3 represents readings from station 3 in the north-slope forest.

TABLE 2.—Evaporimeter losses at various temperatures. Figures represent daily loss in milliliters.

Month	Temperature range	Number readings	Station 1	Station 2	Station 3
April	85° +	7	123.3	74.9	33.7
	71 — 84°	6	55.1	32.9	22.3
	70° + below	0		Rained out	
May	91° +	7	102.0	36.0	24.5
	81 — 90°	4	76.0	31.5	19.4
	80° below	3	74.5	30.3	12.7

velocity as well. Indeed the highest values for both were obtained during a hot, dry and unusually windy period in April. Averages for the entire period reveal that evapotranspiration rates on the southerly prairies are three to four times those of the northerly woods.

It will be noted that the highest rates in Table 2 are for April. This was due both to the abnormally high temperatures recorded during this month and also to the extreme windiness of several periods in this month. Although no anemometer readings were taken, visual estimates showed that these strong winds did not occur in May, even though many of the days were hotter.

The correlation between the evaporimeters and transpirometers was good enough to encourage us to conclude that we might be able to predict transpiration rates from evaporimeters with sufficient data. This would be helpful inasmuch as the latter are much easier to use. Readings from evaporimeters can be taken much more quickly inasmuch as water loss is measured rather than weighed. Surprisingly, the evaporimeters rarely showed water loss by water blowing out, even in high winds. Apparently, the stability in a small container, and the lip of the dish, prevented loss of water from what would appear to be a probable source.

The transpirometers also revealed some problems. The major difficulty with their use, as with the evaporimeters, is that they cannot be used when it rains. At such times rain is added to the containers in different amounts, and the data taken must be discarded. Thus, about a quarter of the readings in this study were wasted.

The transpirometers also revealed the problem of differential growth at the different stations. Those kept in the shade at station 3 eventually became unusable as the grass in them sickened and died, owing to an extended rainy period that kept them over-saturated. It became clear to us that double the number of transpirometers must be prepared, and a rotation scheme utilized in future studies.

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NOTES

GASTROPODS FROM THE TERTIARY DEPOSITS OF NORTHEASTERN COLORADO

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ABSTRACT. — Oligocene Polygyridae, Zonitidae, Endodontidae, Succineidae, Pupillidae, and Miocene Succineidae are reported from northeastern Colorado.

In twenty years of collecting vertebrate fossils in the Tertiary deposits in Logan and Weld Counties, Colorado, I have found several kinds of fossil gastropods. These comments are made in order to record my finds and stimulate interest in these northeastern Colorado fossils.

My search for fossils has been carried on by "prospecting" for larger specimens and "screening and washing" for small items such as teeth. Large specimens such as no. 1700 and 1704 are internal molds found while prospecting. These molds can be recognized only by their shape; therefore the few I found may be the result of properly contrasted light and shadow rather than rarity. On the other hand, many of the minute gastropods have been preserved by crystalline replacement. Consequently in screening and washing about 3800 of these small gastropods (all but fifty are pupillids) have been found.

I have used Pilsbry (1948) as a guide for the family names. Assignment of some of the specimens implies a degree of accuracy not warranted by the condition of the specimens. However, it seems to be the best way to convey an opinion gained from examining the material.

OLIGOCENE TERRESTRAL GASTROPODS

Polygyridae — No. 1705 (Fig. 1E) was found in the lower part of the middle Oligocene beds along with no. 1703 and a few pupillids.

Zonitidae — No. 1703 (Fig. 1C) is one of six similar specimens from the middle Oligocene; the umbilicus is cone-shaped.

Endodontidae — Figure 1A illustrates what I think is an endodontid. Only incomplete internal molds have been found in the massive, fine silt of the lower and middle Oligocene deposits. The dozen found range in size from 10 to 19 mm in greatest diameter.

Succineidae — Oligocene succineids have been found in the Pawnee Buttes area of Weld County. No. 1702 from the middle Oligocene beds is five mm long and three mm in diameter; others (18) are smaller.

Pupillidae — Here, because of preservation, Pupillidae means any small pupillid-like shell. While pupillids are found at all levels in the Colorado Oligocene, the middle part of the middle Oligocene deposits yield the greatest numbers. Nos. 1706-10, 1712-13 (Figs. 1F-1J, 1L-1M) are a few examples of the kinds collected. In these pupillids crystalline replacement is neither confined to the original shape nor necessarily complete and generally fills the body cavity. The area around the aperture seems to be the poorest preserved part of the shell and I fear that cleaning away silt and crystal growth has often created a better lip than was present. In other specimens the crystal growth seems to be excessive. I think the two little "teeth" seen on Figure 1G are calcite growths. I have examined a great number of these specimens seeking teeth such as one seen on some of the

FIGURE 1. — Gastropod Shells from the Tertiary Deposits in Northeastern Colorado. The dimensions recorded with each specimen conform to the orientation seen in the illustrations. Figures A and D are approximately x1.2 and all others are x5.6. All specimens are in the University Museum, Southern Illinois University. A: No. 1700, internal mold, 30.5 x 24.5 mm; center Sec. 30, T. 11 N., R. 53 W., Logan Co.; late Chadronian (lower Oligocene). B: No. 1701, external mold, 2.6 x .8 mm; SE¼ Sec. 36, T. 10 N., R. 59 W., Weld Co.; lower Oligocene. C: No. 1703, external mold, 4.5 x 2.2 mm; NW¼ Sec 10, T. 11 N., R. 54 W., Logan Co.; early Orellan (Middle Oligocene). D: No. 1704, internal mold, 21.0 x 14.4 mm; W½ Sec. 28, T. 12 N., R. 55 W., Logan Co.; upper Miocene. E: No. 1705, external mold, 2.8 x 1.2 mm; data same as "C". F: No. 1706, external mold, 2.3 x 1.8 mm; SW¼ Sec. 12, T. 11 N., R. 54 W., Logan Co.; middle Oligocene G: No. 1707, external mold, 2.7 x 1.8 mm; NE¼ Sec. 17, T. 10 N., R. 59 W., Weld Co.; middle Oligocene. H-K: Nos. 1708-11, external molds (H, 2.5 x 1.6 mm; I, 2.9 x 1.5 mm; J, 2.6 x 1.4 mm; K, 4.7 x 1.5 mm); data same as "F". L-M: Nos. 1712-13, external molds, 4.0 x 1.5 and 4.1 x 1.9 mm; midpoint of west line of Sec. 2, T. 10 N., R. 59 W., Weld Co.; middle Oligocene.

present day specimens. No such structures have been observed, but it is questionable whether or not the teeth would be preserved.

Other Oligocene specimens — No. 1701 (Fig. 1B) is a battered internal cast from the lower Oligocene. Probably the umbilicus was broad and shallow and all the inner whorls are slightly depressed on the opposite side.

No. 1711 (Fig. 1K) and associated material reminds one of a miniature *Euglandia rosea* (Oleacinidae) without striations. The aperture appears to be elongate and flaring. Never-the-less, neither by hand cleaning nor ultrasonic cleaning have I been able to ascertain the true nature of the opening.

Fragments of specimens too incomplete to consider have been found. Tiny cochlear spirals from the skulls of small vertebrates frequently found in the deposits can be most misleading and need careful inspection.

MIOCENE TERRESTRAL GASTROPOD

Sueeineidae — No. 1704 (Fig. 1D) is an average-sized internal mold found

with nine less perfectly preserved specimens in an area about one meter square in beds containing the Vim-Peetz local fauna (Galbreath, 1953) that I consider to be very late Miocene in age. The matrix consists of nodular limy silt, a relic of an old soil horizon — hence terrestral. With only an internal mold I shall call my specimen a succineid because it is terrestral, thus emulating Russell (1938) who thought the aquatic habitat to be a strong argument for deciding that *Pseudosuccinea venusta* from the Oligocene of Park County, Colorado was a lymnaeid.

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CYLINDRODONT RODENTS FROM THE LOWER OLIGOCENE OF NORTHEASTERN COLORADO

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ABSTRACT.—*Pseudocylindrodon* sp. differs from *P. neglectus* in having a narrower incisor, incisor capsule, and mental foramina under the diastema. The geologically youngest specimens of *Cylindrodon fontis* have a weak to strong spur extending posteriorly into the central basin of the cheek teeth.

Remains of cylindrodont rodents are not common in the lower Oligocene deposits in northeastern Colorado. Despite diligent searching, a decade of field seasons passed by before the first specimen was found by Professor Robert W. Wilson's field party in 1958. Of the places where specimens have been found only one, the "Shable Butte" locality (See lists of referred specimens for the localities where cylindrodonts have been found.) can be regarded as productive. I am indebted to Dr. Peter Robinson, University Colorado Museum, for the privilege of collecting at Shable Butte.

I have regarded the Horsetail Creek Beds in northeastern Colorado to be late Chadronian in age (Galbreath, 1953). Within this span of time I think that the "Iliff" and "Molly and Mabel" localities in Logan County are younger than those in Weld County.

Pseudocylindrodon sp.

Referred Specimens: — Right jaw with I, P_4-M_3 no. 10577, Mus. Nat. Hist. Univ. Kansas (KU); fragments of mandible with right M_1-M_3 and left M_2-M_3 no. P-449, Zool. Dept. Co. Illinois Univ. (SIU); "Iliff" locality, Horsetail Creek Beds, W½ secs. 9 and 16, T. 10 N., R. 51 W., Logan County, Colorado. Figure 1 and Table 1.

These two specimens are assigned to *Pseudocylindrodon* because of the short crowns on the cheek teeth and the shape of the lower incisors. The occlusal pattern of SIU P-449 is new enough to demonstrate that the cheek teeth did not have hypsodont crowns like those seen in *Cylindrodon fontis*. Except for the details to be discussed below, the lower incisors match Black's description (p. 10, 1965) of the lower incisors of *P. neglectus*.

The University of Kansas specimen (no. 10577) has features worthy of note. The mental foramina are below the diastema rather than the premolar. An incisor capsule is present on the jaw. Although not shown in Figure 1, a distinct groove separates what remains of the inner wall of the capsule from the surface of the ascending ramus. In both specimens the incisors are narrow; the transverse dimensions being 59 and 62 percent of the anteroposterior dimensions. These ratios are less than the ratios of such dimensions seen in the type and other specimens of *P. neglectus*.

The very narrow and rounded incisor, presence of an incisor capsule, and failure of the mental foramina to shift posteriorly suggest that in these late Chadronian specimens we have the last expression of a line separate from *P. neglectus*. This speculation indicates a species different from *P. neglectus* but I cannot honor my inclination to name a new species because neither specimen is fit to be a type.

Cylindrodon fontis DOUGLASS

Referred Specimens: — RP^4 no. P-447, $R?M^1$ no. P-448, right jaw with P_4-M_3 no. P-450, Zool. Dept. So. Illinois Univ. (SIU); right jaw with I, P_4-M_2, no.

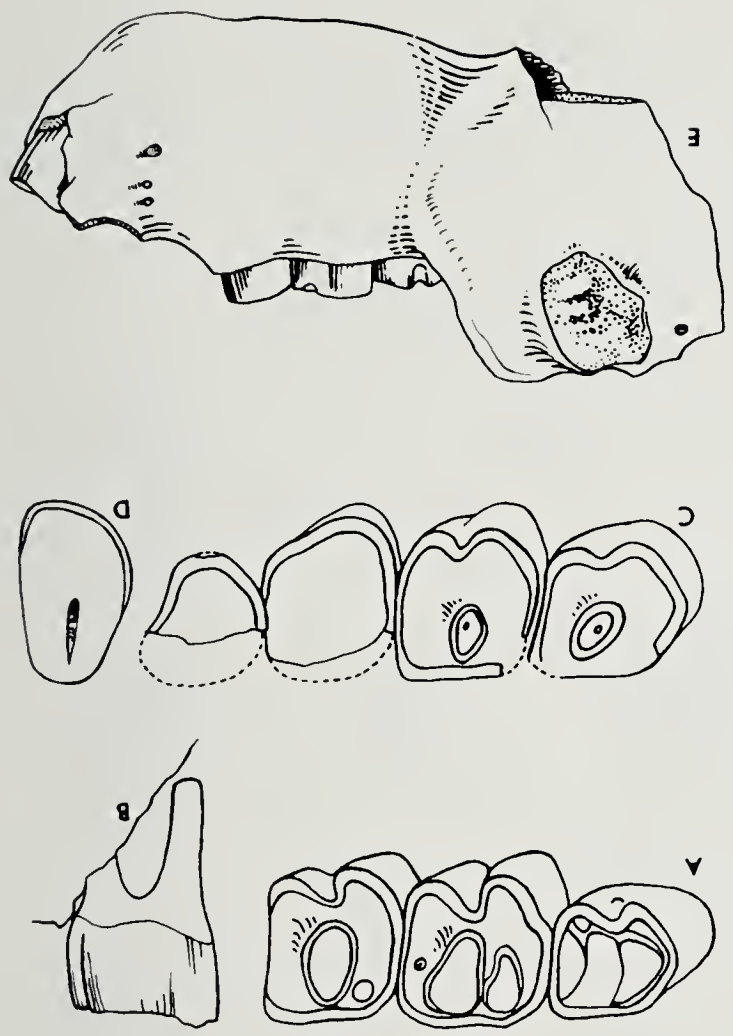

FIGURE 1. — *Pseudocylindrodon* sp. Occlusal view (A) of right M_1-M_3 and anterior view (B) of left M_2 no. P-449, Zool. Dept., So. Illinois Univ. Occlusal view (C) of P_4-M_3, cross-sectional view (D) of incisor, and lateral view (E) of right jaw no. 10577, Mus. Nat. Hist., Univ. Kansas. Views of teeth x6 and view of jaw x3.

11160, Mus. Nat. Hist., Univ. Kansas (KU). Specimens from "Molly and Mabel" locality, Horsetail Creek Beds, N½ sec. 31, T. 11 N., R. 53 W., Logan County, Colorado.

Right M^2, no. P-242 and left jaw with I, P_4-M_3 no. P-451, Zool. Dept. So. Illinois Univ.; "Section 36" locality, Horsetail Creek Beds, SE¼, sec. 36, T. 10 N., R. 59 W., Weld County, Colorado.

P^4 no. 30983, P^4 no. 30991, ?M^1 no. 30985, M^2 no. 30984, left jaw with weathered P_4-M_3 no. 22050, right jaw with I, P_4-M_2 no. 30987, left jaw with P_4-M_2 no. 30988, left jaw with weathered M_1-M_3 no. 30989, and right jaw with I, P_4-M_3 no. 30992, Univ. Colorado Mus. (UCM); "Shable Butte" locality, Horsetail Creek Beds, NE¼, sec. 21, T. 9 N., R. 58 W., Weld County, Colorado. Figure 2 and Table 2.

TABLE 1.—Dimensions (in mm) of Specimens of *Pseudocylindrodon* sp. from the Horsetail Creek Beds of Northeastern Colorado. All anteroposterior and transverse measurements of cheek teeth are at the occlusal surface. KU; Mus. Nat. Hist., Univ. Kansas. SIU; Zool. Dept., So. Illinois Univ.

		KU 10577	SIU P-499 (right jaw)
P_4-M_3	(alv.)	7.90	.....
I	A-p.	2.51[1]	2.43[2]
	Tr.	1.50	1.52
M_1	A-p.	1.90	1.86
	Tr.	.....	2.15
M_2	A-p.	2.00	1.90
	Tr.	2.19	2.10
M_3	A-p.	1.99	1.80
	Tr.	1.72	1.55
Depth of jaw at M_1		7.00	.....
Height of left M_2		.....	3.00

[1] Deep to alveolus.
[2] Under M_1.

Only the specimens of *Cylindrodon fontis* from the "Molly and Mabel" locality need comment. A sharp spur, arising from the metalophid, extends posteriorly into the central basin of the lower cheek teeth. The feature is present but not well-developed on KU 11160. No other cylindrodont teeth that I know of show this spur. The presence of the spur takes on additional interest when one realizes that these specimens came from beds that could be regarded as intermediate in age between Chadronian and Orellan times.

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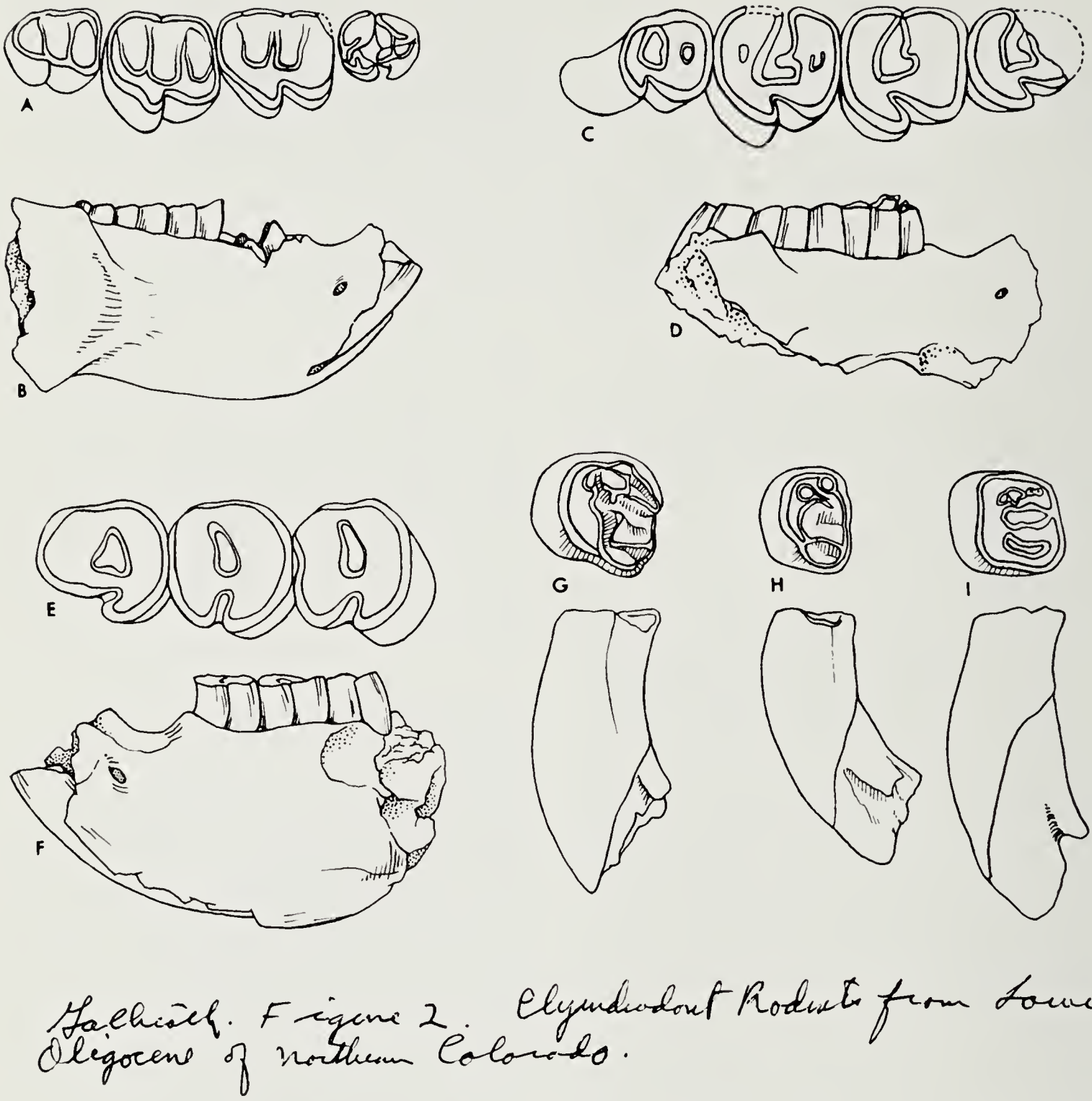

Hatcher. Figure 2. Clyndrodont Rodents from Lower Oligocene of Northern Colorado.

FIGURE 2. — *Cylindrodon fontis* Douglass. Occlusal view (A) of P_4-M_3 and lateral view (B) of right jaw no. 30992, Univ. Colorado Mus. Occlusal view (C) of P_4-M_3 and lateral view (D) of right jaw no. P-450, Zool. Dept., So. Illinois Univ. Occlusal view (E) of P_4-M_2 and lateral view (F) of left jaw no. P-451, Zool. Dept. So. Illinois Univ. Occlusal and anterior views (G) of P^4 no. P-447, (H) ?M^1 no. P-448, and (I) M^2 no. P-242, Zool. Dept., So. Illinois Univ. Views of teeth x6 and views of jaws x3.

TABLE 2.—Dimensions (in mm) of Specimens of *Cylindrodon fontis* from the Horsetail Creek Beds of Northeastern Colorado. Except where noted, all anteroposterior and transverse measurements of cheek teeth are at the occlusal surface. KU; Mus. Nat. Hist., Univ. Kansas. SIU; So. Illinois Univ. UCM; Univ. Colorado Mus.

		SIU P-447	UCM 30983	UCM 30991	SIU P-448	UCM 30985	SIU P-242	UCM 30984
P^4	A-p.	2.30	1.73	1.85	.....	.....	.....	.....
	Tr.	2.40	1.80	1.70	.....	.....	.....	.....
	Height[1]	5.23	4.30	3.60	.....	.....	.....	.....
?M^1	A-p.	.....	.....	.....	1.88	1.76	.....	.....
	Tr.	.....	.....	.....	1.74	1.60	.....	.....
	Height	.....	.....	.....	4.60	3.40	.....	.....
M^2	A-p.	.....	.....	.....	.....	.....	1.82	1.90
	Tr.	.....	.....	.....	.....	.....	1.74	2.00
	Height	.....	.....	.....	.....	.....	5.45	4.60

		KU 11160	SIU P-450	SIU P-451	UCM 22050	UCM 30987	UCM 30988	UCM 30989	UCM 30992
P_4-M_3 (Alv.)		.....	.....	.....	.....	.....	.....	.....	7.50
Depth of jaw at M_1		.....	.....	8.+	7.21	7.20	.....	.....	.....
I	A-p.	2.15[2]	.....	2.45[2]	.....	2.04[2]	.....	.....	1.90[3]
	Tr.	2.06	.....	2.41	.....	1.95	.....	.....	1.79
P_4	A-p.	2.10	.....	2.40	.....	2.25	1.86	2.00	1.68[4]
	Tr. (a.)	1.27	.....	1.55	.....	.....	1.22	.....	1.40
	Tr. (p.)	1.73	2.11	2.19	.....	1.77	1.54	.....	1.60
M_1	A-p.	2.32	2.35	2.30	.....	1.87	1.85	2.00	2.10
	Tr.	1.93	2.26	2.37	.....	2.10	1.81	.....	1.54
M_2	A-p.	2.23	2.35	2.15	2.09	1.87	1.99	1.72[5]	1.98
	Tr.	1.88	2.10	2.37	.....	2.10	1.76	.....	1.55
	Height	.....	.....	.....	.....	.....	4.50	5.50	.....
M_3	A-p.	.....	1.68	.....	1.78	.....	.....	.....	1.67
	Tr.	.....	1.49	.....	.....	.....	.....	.....	1.20

[1] Here and elsewhere, height is dimension from occlusal surface to end of root.
[2] Dimensions deep to alveolus.
[3] Dimensions at alveolus.
[4] Dimensions of visible part of tooth.
[5] Approximate dimension.

THE GENUS CARDUUS IN ILLINOIS

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ABSTRACT. — Distinguishing characters and distributional data for *Carduus nutans* var. *leiophyllus* and *Carduus acanthoides* are given.

Carduus nutans L. var. *leiophyllus* (Petronic) Arenes, a native of Europe, Asia, and north Africa, is a recent introduction in Illinois where it occurs locally along roadsides, in pastures, and in waste ground throughout Illinois (Fig. 1). The first report of *Carduus nutans* in Illinois was by Ries (1939) who reported it from Starved Rock State Park, LaSalle County. Ries' report was based on a misidentification of *Cirsium vulgare*, thus the first authentic report was probably that of Jones (1945). The earliest collection seen by the author is from Piatt County, collected in 1930 by L. R. Tehon.

The seeds of this species, having a large pappus composed of capillary bristles, are well adapted to wind dispersal. It is fast becoming a pestiferous species in several areas in Illinois and because of its efficient dispersal method, there is a great likelihood of it becoming a cosmopolitan weed. It is avoided by grazing animals because of the spiny nature of the leaves, thus it invariably produces seed unless prevented in doing so by man.

Carduus nutans has been reported by Ries (1939), Jones and Fuller (1955), Mohlenbrock and Voigt (1959), Winterringer and Evers (1960), and Jones (1945, 1950, 1963) as occurring in Illinois. The author has examined the Illinois material in the herbaria at the University of Illinois (ILL), Southern Illinois University (SIU), Eastern Illinois University (EIU), the Illinois State Museum (ISM), the Field Museum of Natural History (F), and the Illinois Natural History Survey (ILLS) and is of the opinion that it should all be treated as var. *leiophyllus*. No specimens of *Carduus nutans* var. *nutans* have been seen by the author, so it is excluded from the Illinois flora.

Carduus nutans var. *nutans* has pubescent leaves, heads 1.1-3.9 cm in diameter, and phyllaries with white cobwebby hairs while var. *leiophyllus* has glabrous or nearly so leaves, heads 4.4-5.3 cm in diameter, and the phyllaries glabrous except for minute hairs on the upper surface.

SPECIMENS EXAMINED: ALEXANDER CO.: S of McClure, *Winterringer 3064* (ISM), *7128* (ISM, F); N of McClure, *Winterringer 10302* (ISM), *Evers 53293* (ILLS); 0.8 mi. N of McClure, *Winterringer 10759* (ISM); Along Route 3, 0.25 mi. N of McClure, *Thieret 3216* (F). CHAMPAIGN CO.: 5 mi. S of Gibson City, *Winterringer 13612, 13639, 13640* (ISM). COLES CO.: Old Ashmore road, *Thut s.n.* (EIU). COOK CO.: S of Barrington, *Evers 44801* (ILLS); Along Shoe Factory road, E of Sutton road, 4.5 mi. N of Bartlett, *Bennett 7115* (F, ILL, ILLS); *7067* (F). FRANKLIN CO.: 0.5 mi. N of Sesser, *Hogancamp s.n.* (ISM). JO DAVIESS CO.: Between Galena and Hanover, *Wunderlin & Chapman 394* (SIU). KNOX CO.: 4 mi. S of Alpha, *Chase 16095* (ILLS, ILL). LAKE CO.: 3 mi. NW of Libertyville, *Richardson 499* (SIU); Near Waukegan, *Brown s.n.* (ILL). LEE CO.: Dixon, *Barnes s.n.* (ILLS). MADISON CO.: E of Bethalto, *Winterringer 16095, 16096, 16097* (ISM); Near Poag, *Winterringer 22072, 22073* (ISM). MC HENRY CO.: N of Ringwood, *Evers 81022* (ILLS). MERCER CO.: N of New Boston, *Evers 83847* (ILLS); W of Joy, *Winterringer 13869, 13870, 13871* (ISM); E of Joy, *Wunderlin & Chapman 273* (SIU); 7 mi.

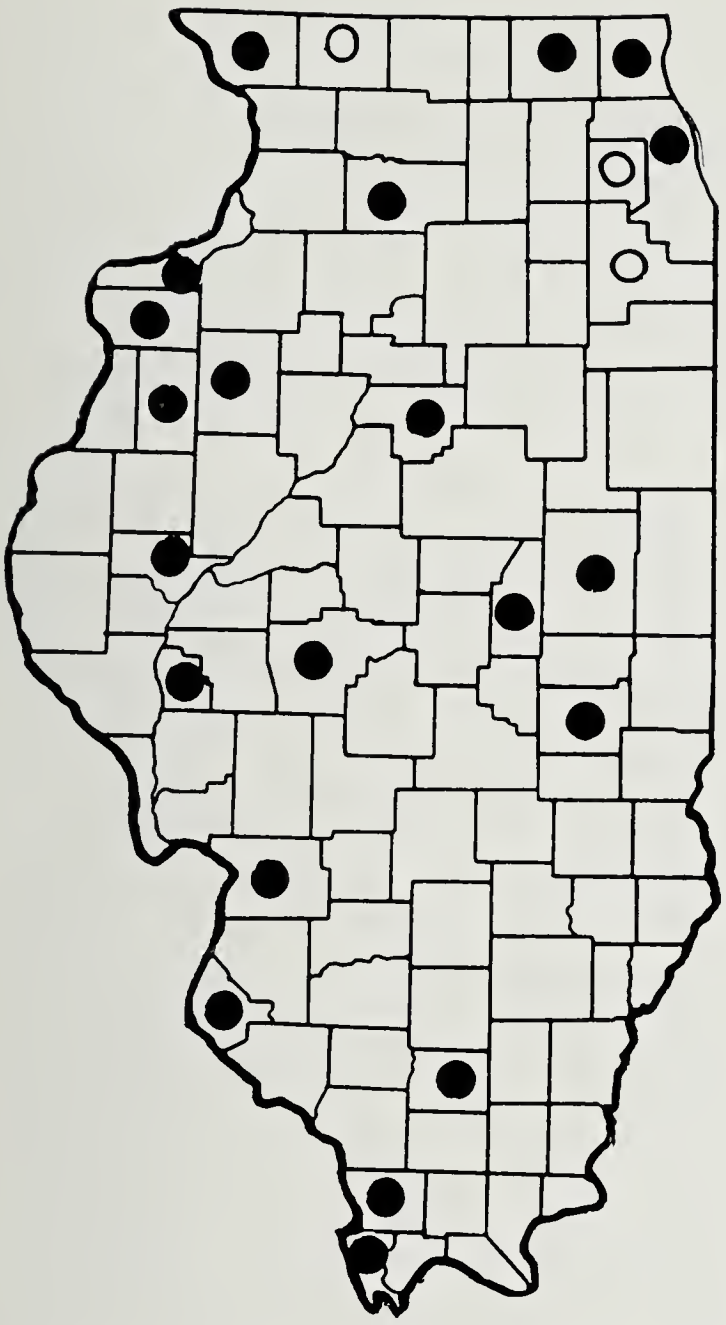

FIGURE 1. — Distribution of the species of *Carduus* in Illinois by counties (● = *C. nutans* var. *leiophyllus*; O = *C. acanthoides*).

N of Little York, *Wunderlin & Chapman 106, 109* (SIU); 2 mi. W of Joy, *Wunderlin & Chapman 107, 108* (SIU). MONROE CO.: E of Fountain, *Evers 83408* (ILLS). PIATT CO.: 3 mi. S, 1 mi. W of Seymour, *Tehon s.n.* (ILLS). ROCK ISLAND CO.: SW of Illinois City, *Evers 83848* (ILLS). SANGAMON CO.: Near Springfield, *Springfield Nature League 11779* (ISM), *Bonney s.n.* (ILL). SCHUYLER CO.: SW of Browning, *Winterringer 19501, 19502* (ISM). SCOTT CO.: Merritt, *Rexroat 3835, 3836* (ISM); W of Winchester, *Evers 11980* (ILLS). UNION CO.: Between Ware and Reynoldsville, *Winterringer 1941* (ISM, ILL); 2 mi. S of Reynoldsville, *Winterringer 4480* (ISM, ILL); Union County Refuge, *Dolbeare 849* (ISM). WARREN CO.: Near Lake Warren, *Wunderlin & Chapman 110* (SIU). WOODFORD CO.: Near Congerville, *Chase 18402* (ILLS, ILL); W of Congerville, *Evers 83784* (ILLS).

Carduus acanthoides L., a native of Europe and southern Russia, is also a recent introduction in Illinois. It was first reported as occurring in Illinois by Winterringer & Evers (1960) who reported it from Stephenson County. As of this publication it is known from three counties in northern Illinois (Fig. 1). It also has the potential of becoming a noxious weed.

It is readily distinguished from *Carduus nutans* var. *leiophyllus* in having the involucre 1.5-1.7 cm high, heads clustered, and peduncles winged immediately below the heads while *Carduus nutans* var. *leiophyllus* has the involucre 2.8-3.0 cm high, heads solitary, and the peduncles wingless immediately below the heads. It resembles *Cirsium vulgare* and *Cirsium palustre* but differs in having capillary pappus bristles, while *Cirsium* has plumose bristles.

SPECIMENS EXAMINED: DU PAGE CO.: Along Green road, 1 block S of Hobson road, S of Lisle, *Swink s.n.* (ILLS); S of city limits of Lisle, *Evers 77177* (ILLS); Wheaton, *Moffatt s.n.* (ILL). STEPHENSON CO.: s. loc. *County Agent s.n.* (ILL). WILL CO.: On an island in the DuPage River at Hammel Woods Forest Preserve, W of Joliet, *Schulenberg s.n.* (SIU).

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NEW AND UNUSUAL GRASS AND SEDGE RECORDS FOR ILLINOIS

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ABSTRACT. — Four grasses and four sedges are reported for the first time from Illinois. In addition, range extensions of several rare species are recorded.

Intensive efforts in exploring Illinois for new records of vascular plants have been met with several interesting grasses and sedges previously unknown from the state, and others representing significant range extensions in Illinois. Appreciation is accorded Dr. Glen S. Winterringer of the Illinois State Museum and Dr. Norlan Henderson of the University of Missouri at Kansas City who made available some of their collections.

GRASSES AND SEDGES NEW FOR ILLINOIS

Eulalia viminea (Trin.) Kuntze. This grass, a native of Asia, has been reported as locally adventive in a few eastern states. A member of tribe Andropogoneae, it differs from all other Illinois representatives of this tribe by its decumbent, annual habit. The junior author was pleasantly surprised to find this low creeping species in waste ground near an abandoned house on the Ohio River floodplain. It was first collected two miles south of Bay City, Pope County, September 21, 1967, *J. Schwegman 1454*, and later along the Ohio River at Fort Massac State Park, Massac County, October 28, 1967, *J. Schwegman 1503*.

Sitanion hystrix (Nutt.) J. G. Sm. This species, called Squirreltail, has a resemblance to *Hordeum jubatum* and was found growing with this species in Illinois. *Sitanion*, however, has spikelets in twos, while *Hordeum* has spikelets in threes. The occurrence in Illinois along a railroad would indicate that this western species is adventive in Illinois. Mason County: cinders on abandoned railroad right-of-way, October 17, 1966, *R. T. Rexroat 10397* (ISM, SIU).

Andropogon saccharoides Sw. In the little village of Thebes, in Alexander County, not more than 300 yards from the Mississippi River, occur several plants of *Andropogon saccharoides*. These plants, conspicuous in September because of their silvery inflorescence, are growing in a vacant field. This species has been known previously from southern Missouri. The original collection from Illinois was made in 1966. This is the only species of *Andropogon* in Illinois with a panicle. Alexander County: vacant lot, Thebes, October 4, 1966, *G. S. Winterringer 23679* (ISM, SIU).

Andropogon praematurus Fern. This interesting plant was described by Fernald from dry fields in states adjacent to the Atlantic Ocean. It differed from all other species of *Andropogon* by its very early flowering time (nearly two months before the similar *A. scoparius*) and its shorter racemes bearing only single lateral pedicellate spikelets. It subsequently has been shown that this plant is apparently a smut-infected form of some species of *Andropogon*. It is true that the Illinois collection reported here is full of smut. The only species near to this is *A. scoparius*. It is interesting to speculate that the effect of the smut on *A. scoparius* is manifested in the morphology whereby a normal paired lateral spikelet condition is reduced to a single lateral spikelet. The collection data for the specimen reported here are: field, near Long Spring, Pope County, July 3,

1967, *J. Schwegman & R. H. Mohlenbrock 14521.*

Cyperus compressus L. The natural range of this species is the southern United States and tropical America, although it is often naturalized throughout the eastern United States. It was to be expected in Illinois since it is known from Missouri. Lawrence County: in drainage ditch, along U. S. Route 50, nine miles west of Lawrenceville, September 1, 1964, *Norlan Henderson 64-549a.*

Carex incomperta Bickn. This sedge of swamps and bogs is of scattered local occurrence in the midwest. In Illinois it is a typical plant of sandy seep springs in the Cretaceous Hills section of southern Pope County. It grows with such rare Illinois species as *Woodwardia areolata, Bartonia paniculata,* and *Isotria verticillata.* This species belongs to § Stellulatae, a section represented in Illinois also by *C. interior* and *C. sterilis.* From *C. interior, Carex incomperta* differs by its broader perigynia whose beak is more sharply bidentate. From *C. sterilis,* it differs by its broader and more strongly nerved perigynia. The original Illinois collection was made four miles southwest of Bay City, Pope County, on May 9, 1967, *J. Schwegman 1166.*

Carex reniformis (Bailey) Small. Fernald (1950) gives the northern limit of range for this sedge as southeast Virginia, Arkansas, and Oklahoma. This *Carex* is a plant of cypress swamps and wet ditches. It is found in both of these habitats at the Mermet Wildlife Refuge in Massac County, where it is associated with such Coastal Plain species as *Paspalum lentiferum.* This species is one of many Illinois members of *Carex* § Ovales. It is easily distinguished by its nearly orbicular perigynia which are at least 3.5 mm broad. Our specimen was collected May 22, 1967, by John Schwegman *1194.*

Carex abscondita Mackenz. This is the verification of the report of this species from Illinois when it first appeared in a list of plants collected at Beall Woods, Wabash County (Ashby & Ozment, 1967). This species belongs to § Laxiflorae. It has extremely acute-angled perigynia subtended by blunt scales; the uppermost bract is inflated into a spathelike structure. Wabash County: rich hardwood forest, Beall Woods, four miles south of Mt. Carmel, May 17, 1963, *J. E. Ozment* s.n.

New Distributional Records of Illinois Grasses and Sedges

Aristida curtissii (Gray) Nash. This unusual grass in Illinois has been known previously only from Ogle and Mason Counties. It has now been collected along the CB&Q railroad at the Mermet Conservation Area, Massac County, on October 3, 1967, by John Schwegman *1487.*

Carex bromoides Schkur. A sedge of low swampy woods, this *Carex* was found in a large stand in woods kept moist by nearby springs. *Carex incomperta* grows in the adjacent springs. *Carex bromoides* was collected three-and-one-half miles southwest of Bay City, Pope County, on May 20, 1967, *J. Schwegman 1189* (SIU).

Carex oxylepis Torr. & Hook. This species was previously known in Illinois only from Union County, where it was discovered by Mohlenbrock and Voigt. It has now been found in low woods along the Cache River, one mile northeast of Belknap, Johnson County, on May 8, 1967, *J. Schwegman 1160* (SIU).

Carex straminea Willd. (= *C. richii* (Fern.) Mack.). A collection of this species from Bond County, Illinois, has been detected during a study of *Carex* on deposit in the herbarium of Southern Illinois University. This collection marks the second known for this species from Illinois, the original collection having been made by Mead during the latter half of the nineteenth century from Menard County. The collection reported here was made in Section 7, T 5 N, R 3 W, east of Greenville, in low ground between highway and railroad on May 27, 1952, by *W. Bailey* and *J. Swayne 2386.*

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DETECTION OF PLANT VIRUS SYMPTOMS WITH INFRARED PHOTOGRAPHY

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ABSTRACT. — A technique for taking close-up infrared photographs, was developed which could prove to be a valuable tool in the study of virus and other types of plant disease. It was shown that infection by Tobacco Ringspot Virus — Strain 3 resulted in reduced infrared reflectance in bean, cucumber, tobacco and snapdragon leaves. The lesions produced by TRSV-3 did not reflect infrared but appeared white to yellowish in infrared film. Occasionally the altered infrared reflectance could be detected before visible signs of infection appeared on the leaves of inoculated plants which suggests the possibility of early symptom detection.

Virus symptom expression is influenced by a wide variety of factors including temperature, condition and age of the host. In any study of virus infection it is important to know the specific symptoms produced as well as their time of appearance. The use of infrared photography as a method of virus symptom detection was first mentioned by Bawden (1933) when he observed that tobacco necrosis virus produced local lesions capable of reflecting infrared radiation. It wasn't until the 1950's however, that efforts were made to study the infrared reflectance phenomena on a broader scale (Knipling, 1967).

Infrared Aero Film, has been developed for this purpose; (Fritz, 1967; Colwell, 1968; and Cassida, 1968). From its use we have learned that healthy leaves generally have a high infrared reflectance and a low visible light reflectance. Physiological disturbances in leaves nearly always cause an increase in visible light reflectance, but the changes in infrared reflectance are variable (Knipling, 1967). In most cases, differences seen with Infrared Aero Film are also observable visibly or on conventional color photographs of the same foliage. The real value of the film is its "enhancement of ordinarily slight color differences that otherwise might be questionable or completely overlooked (Knipling, 1967)." According to Park (1967), infrared color changes may occur before other changes (symptoms) become apparent. Knipling (1967) stated that the chlorophylls are highly transparent to infrared radiation. Since the cuticle and the epidermis are almost completely transparent to visible and infrared wavelengths, very little of the incident radiation is reflected directly from the external surface of the leaf. The exact nature of the internal reflectance is not known but it may be related to the presence of sub-cellular organelles or cell wall-air cavity interfaces.

Viruses are known to cause symptoms such as chlorosis and necrosis which can be related to internal changes in plant leaves (Bald, 1966). Yellowing of beet leaves by beet yellows virus for example involves the probable interference with plastid replicating machinery (Cronshaw, Hoefert and Esau, 1966). The production of chlorophyll and cytochromes in plant leaves is affected by the multiplication of subcellular organelles and the development of their complement of synthetic enzymes. In short, the parasitic relationship of viruses within plant tissue might be predicted to produce a change in the infrared reflectance of the infected host.

Few attempts with close-up infrared photography to study plant disease have been successful (Fritz, 1967). Success

here would provide a valuable tool for the study of plant virus infection and also add to the growing fund of information on altered infrared reflectance under prescribed conditions. Thus the purpose of this investigation was to examine the effect of Tobacco ringspot virus strain 3 infection on the infrared reflectance of selected plants.

Materials and Methods

Seeds of *Nicotiana tabacum* L. var. Xanthi, nc., a systemic host for TRSV-3, were germinated in a mixture of one-half peat (Michigan) and one-half sand (Ottawa silica sand, fine). These plants were watered with a balanced nutrient solution. When the seedlings reached 6-8 inches in height, they were inoculated with TRSV-3. Additional inoculations were made every 14-21 days to new Xanthi tobacco seedlings to maintain a constant source of TRSV-3. According to deZeeuw (1967), Takahashi's *Nicotiana tabacum* L. var. Xanthi, nc. is best used since tobacco mosaic Virus (TMV) produces only local symptoms and is eliminated as a source of contamination. Xanthi tobacco does carry cucumber mosaic virus but these symptoms are similar to TMV (Dickson, 1956).

Seeds of bush snap bean (*Phaseolus vulgaris* L.) and cucumber (*Cucumis sativus* L. var. Marketer), both systemic hosts for TRSV-3, were germinated in heat sterilized vermiculite. When they began to show their first true leaves (after 8-12 days) the cucumbers were transplanted into 3 in. diameter plastic pots (3 seedlings per pot) and the beans into 6 in. diameter clay pots (5 per pot). Each pot was fitted with a glass wool pad at the bottom (Foster, 1967) which allowed excess water to drain but retained the fine, heat sterilized Ottawa silica sand used as the potting medium. The small pots were used to conserve sand and reduce exposure of the potting medium when photographs were made (Park, 1967).

After transplanting, these plants received a balanced nutrient solution every three days for about two weeks. They were then inoculated with TRSV-3. Inoculations were made to the cotyledonary leaf or trifoliate leaves of bean and to the cotyledons or first true leaves of cucumber.

Two snapdragons were also studied. Seeds of *Antirrhinum majus* L., which has a diploid chromosome number of 16, and *Antirrhinum tetra* which has a chromosome number of 32, were germinated in a heat sterilized mixture of one-half peat and one-half vermiculite. The seeds were sprinkled on the surface of the soil in 8″ x 8″ x 2.25″ plastic flats and not transplanted. The snapdragons were also watered with a balanced nutrient solution and inoculated in the same manner as the bean, cucumber, and tobacco plants.

Virus inoculations were made using a crude extract obtained by grinding infected leaves in a sterilized mortar and pestle. The inoculum was applied with a T-bar glass inoculator (deZeeuw and Timmer, 1964) to leaves lightly dusted with 600 mesh carborundum. After all inoculations were made, the leaves were rinsed gently with distilled water (deZeeuw, 1967) or 0.01 M phosphate buffer (Roberts and Corbett, 1961).

The plants were kept in a greenhouse constructed of scrim glass. Two banks of four 40 watt fluorescent tubes plus a bank of five 150 watt incandescent bulbs were arranged to give 900-1000 footcandles of light when the plants were within 1.5 feet of them. An 18 hour photoperiod was used throughout the experiment.

Steam sterilization of apparatus, soil and solutions in addition to rinsing or wiping with 40% formaldehyde, when appropriate, was used to safeguard against contamination. The greenhouses were kept free of aphids and other insects that might have served as vectors for virus transmission.

The infrared photographs were taken with a Retina reflex camera to which was attached a Kodak R1: 4.5 magnifying lens for close-ups within 11 3/16 inches. When taking pictures in sunlight, a Wratten No. 12 yellow filter was attached to the front of the camera lens. A Kodak CC20C gelatin filter plus a Corning C.S. 1-59 glass filter were used, in addition to the above, for photography with 3400° Kelvin photoflood lights. The yellow filter was used to withhold blue light to which the film emulsion layers are sensitive. The two additional filters provided the proper color balance in infrared pictures taken with photofloods.

Optimum methods for the use of Kodak Ektachrome Infrared Aero Film, Type 8443 (35mm — 20 exposures) have been described in great detail by Fritz (1967) and the package directions are relatively clear. The film has a very small latitude of only ± ½ f/. Exposures for best results were bracketed

around f/ 5.6 at 1/500 second (Fritz, 1967).

Two photoflood lights were placed, one on either side of the tripod held camera, at a 35-40° angle with respect to the subject. A white bedsheet served as a backdrop (Gibson, Buckley and Whitmore, 1965). A piece of yellow posterboard, that appears white in infrared, was used as the primary background for the photographs which were taken vertically. Photographs of all plants were taken (1) just prior to inoculation; (2) 24 hours after inoculation, as suggested by Park (1967); and (3) every 3-4 days for about two weeks.

For comparative purposes, regular color pictures were taken occasionally with Kodak High Speed Ektachrome Film, Type B (35mm). The set-up was similar except that 3200°K, tungsten flood lights replaced the 3400°K photofloods and a white posterboard background was used instead of yellow. No filters were placed in front of this camera lens but close-ups could be obtained by adding a Tiffen Photar magnifying lens with the appropriate adapter ring. The best results occurred with a camera setting of 1/50 second and f/ 4.7 as determined by bracketing.

Films were developed using Kodak process E-2. It is also recommended that the film be stored in a refrigerator, allowed to warm to room temperature before using and be developed as soon as possible after the last picture is taken (Gibson et al., 1965).

A record was made of the time and character of the symptoms which developed in these experimental plants.

Results and Discussion

The reduced infrared reflectance of infected tobacco leaves was not detectable until 3-4 days after inoculation. This differs from the results obtained by Park (1967) who found that when pinto bean leaves were inoculated with TMV they produced detectable symptoms within 24 hours.

The symptoms observed in normal color film corresponded closely with differences seen using infrared film. However, the infrared film in most cases enhanced these differences. Newly formed leaves had high infrared reflectance until the virus spread into their tissues.

The symptoms of tobacco ringspot virus-3 infection in snapdragons were similar in some respects to those in Zanthi tobacco. The lesions began as minute grayish spots which grew into the characteristic ringspots. Gradually concentric rings of necrotic tissue appeared until the whole leaf became necrotic and died. Symptoms developed at about the same time in both species of snapdragon. However, they became systemic sooner and also killed the leaves faster in *Antirrhinum majus* L. (diploid) when compared to the hybrid *Antirrhinum tetra* (tetraploid). The apparent effect of the tetraploid chromosome condition was to decrease susceptibility to TRSV-3 infection.

Table 1.—Average Time from inoculation until detection of Tobacco Ringspot Virus Strain 3 systemic symptoms.

	Visual Observation	*Infrared Film*
Phaseolus vulgaris	8 days	7-8 days
Cucumis sativus	10 days	9-10
Nicotiana tabacum var. Xanthi, nc	5	5-6
Antirrhinum majus	4	2-3
Antirrhinum tetra	4	2-3

The lesions produced in snapdragon were only visible upon close examination. With infrared photography, however, the infection could be detected easily at a distance of 3 feet. The infected leaves were pink as compared with the red color of healthy leaves in infrared photography. The decreased infrared reflectance was measurable one day earlier (Table 1) than visible symptom manifestation. As in the case of tobacco, the reduced infrared reflectance was localized only in the region of lesions as they formed.

Tobacco ringspot virus strain 3 produced similar lesions in *Phaseolus vulgaris* L. var. bush snap bean and *Cucumis sativus* L. var. Marketer. Visible symptoms appeared, on the average, within 8.4 days on bean and 9.8 days on cucumber leaves. However, some reduction in infrared reflectance was observed sooner (within 24 hours after inoculation) in cucumber than in bean (within 48 hours after inoculation). Significant reduction of infrared reflectance as detected by infrared film was not observed until 1-2 weeks after inoculation which coincided with near optimal appearance of visible symptoms. It was generally

observed that the local lesions themselves did not reflect infrared radiation but appeared as white or yellow rings. The greatly reduced infrared reflectance of highly infected leaves resulted from the cumulative effect of leaves having 100-200 lesions each.

The main consideration of this investigation was the possibility of detecting virus infection before symptoms became visible to the unaided eye. The reduced infrared reflectance that was observed in this investigation was clearly associated with infection by tobacco ringspot virus strain 3. Other investigators, using different viruses and host plants have observed an increased infrared reflectance. Whether the reflectance of infrared wavelengths increased or decreased, it was almost always related to an unhealthy condition of the plant (Knipling, 1967). Therefore, altered infrared reflectance can be used as a means of early symptom detection by both remote sensing and close-up photography.

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ELECTRON MICROSCOPY TECHNIQUE: QUICK DIP METHOD FOR PREPARING PLANT INTRACELLULAR ULTRAPARTICULATES

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ABSTRACT. — Dip preparation, a quick, easy method of preparing electron microscope mounts of plant material, provides a means of detecting and characterizing ultraparticulates in the sap of healthy and diseased plant tissues. Essential necessary materials and preparatory instructions are given; usefulness, advantages and limitations of the method are discussed.

The preparatory technique described here embodies some modifications of the technique developed by Dr. J. Brandes in Germany (1957) for the detection and characterization of viral particles in the sap of infected plants.

The basics of the technique was obtained in a two-month period of study with Dr. Brandes in the Central Electron Microscope Laboratory of the University of Illinois. The modifications have evolved from frequent use of the technique in plant virus research and instruction in a laboratory course in electron microscopy.

The technique, requiring simple inexpensive equipment, is presented to encourage the use of a quick, easy, direct method for the detection and characterization of viruses or other ultramicroparticulates in the cell sap of plant tissues.

MATERIALS AND METHODS

Materials necessary for the technique (illustrated in Figure 1) are:

Alcohol lamp
Single edge razor blades
Forceps (fine and coarse)
Micropipettes
Double distilled water
Negative stain (uranyl acetate, 1%; uranyl formate, 1%; or phosphotungstic acid, 1% adjusted to pH 6.7)
Electron microscope grids coated with substrate film (Formvar, Collodion, or carbon)
Plant material (root, stem, leaf, blossom, et al.)

The methodology consists of the following procedures:

Place a droplet of double distilled water or appropriate heavy metal negative stain in double distilled water on a coated microscope grid. With a cooled, flame-sterilized razor blade excise a small portion of plant material and quickly touch the freshly cut surface of the sample to the surface of the liquid droplet on the grid. Due to the interface exchange between the two liquid surfaces (i.e. surface of droplet on the grid and that of the freshly exposed plant cells) cellular components are quickly drawn into the liquid on the grid. Therefore, it is important to avoid excessive movement or extended contact of the plant sample with the droplet of liquid on the grid. A light, steady touch to the surface of the liquid will produce much better preparations than immersing and swishing the plant sample in the liquid.

Preparations made in droplets of water are covered to prevent contamination from air particles and allowed to dry down. If the dip is made in negative stain the excess liquid is withdrawn after 60 sec by capillarity with a piece of filter paper.

Since viral particles are transparent to electrons their contrast against the background matrix must be enhanced before viewing them in the electron

FIGURE 1. — Materials necessary for dip preparations of plant samples. From left to right according to use: Alcohol lamp for sterilizing single edge razor blades. Vial containing 5 lambda capillary micropipettes above a pipette assembled in glass holder with delivery bulb. Electron microscope grids coated with supporting film of Formvar, Collodion, or carbon on glass microscope slide. Bottle of double-distilled water to be put dropwise onto each grid with a micropipette. Freshly razor-excised sample from systemically diseased leaf of *Nicotiana tabacum*, held in fine forceps, ready to be touched to droplet of water on the grid. Other diseased leaves are *Chenopodium quinoa* with viral chlorotic local lesions and *Zea mays* with systemic yellow streaks.

microscope. For dip preparations made in water, viral contrast is enhanced by depositing a fine film of heavy metal (platinum, palladium, etc.) vaporized at a desired angle onto grids in a high vacuum, high voltage evaporator. This produces a shadowed effect since the metal is deposited against the side of the particulates on the grid in line with the path of the evaporated metal. Thus the sides of the particle away from the path of the metal and the grid area in the "shadow" of the particle are not plated with the metal. Particles in dip preparations made in heavy metal negative stains are seen as white rods embedded in a dark matrix of stain because the metal does not penetrate into the structure of the particle, thus leaving the particle transparent to the electrons and the matrix and intraparticulate channel, if present, opaque to the electrons.

Results and Discussion

Representative micrographs from dip preparations of viral diseased plants made in water and negative stains are presented in Figure 2.

In contrast to the usual methods of viral bio-assay, dip preparations provide a rapid method of assaying plants for assessment of their viral content and viral distribution within the plant. General morphology of the particles is seen and identification of the virus can usually be made quickly without employing time-consuming differential host symptom studies. Ultrastructure of the individual particles are brought out when the dip is made in a droplet of negative stain (i.e. uranyl acetate, uranyl formate, or phosphotungstic acid) on a carbon-coated grid. Particles seen in the mounts prepared by this technique are likely to be near their configuration in nature since the particles are not subjected to alteration by usual purification procedures. Particle form is readily determinable by viewing fields in the microscope or negatives of the fields. After careful calibration of the microscope and photographic enlarger, particle dimensions (length and diameter) are determinable by measuring the enlarged images of the particles projected from the negatives onto white paper in a dark room. From the number of particles observed in these fields a quick estimation of viral concentration can be made rather than using the time-consuming local lesion assay as a measure of viral concentration.

The technique has additional advantages over other assaying procedures. Plant material collected in the field or greenhouse can be returned to the laboratory for preparation with little or no change in tissue metabolism. Since the preparation is simple and easy no great skill or training is necessary for mastery of the technique. Only requirement is a fairly steady hand. The tools required are readily available and inexpensive. The outstanding advantage is the good quality of preparations that can be made in a short time — from the living plant to the microscope in one to two hours.

On the other hand, there are certain limitations to the use of the technique in virology. Since some non-viral cellular components are spherical, dip preparations are meaningful only in the identification of rod-shaped viral particles. The degree of senescence of the plant part used for sampling and carefulness with which the dip preparation is done determines the amount of extraneous cellular components on each grid. Fi-

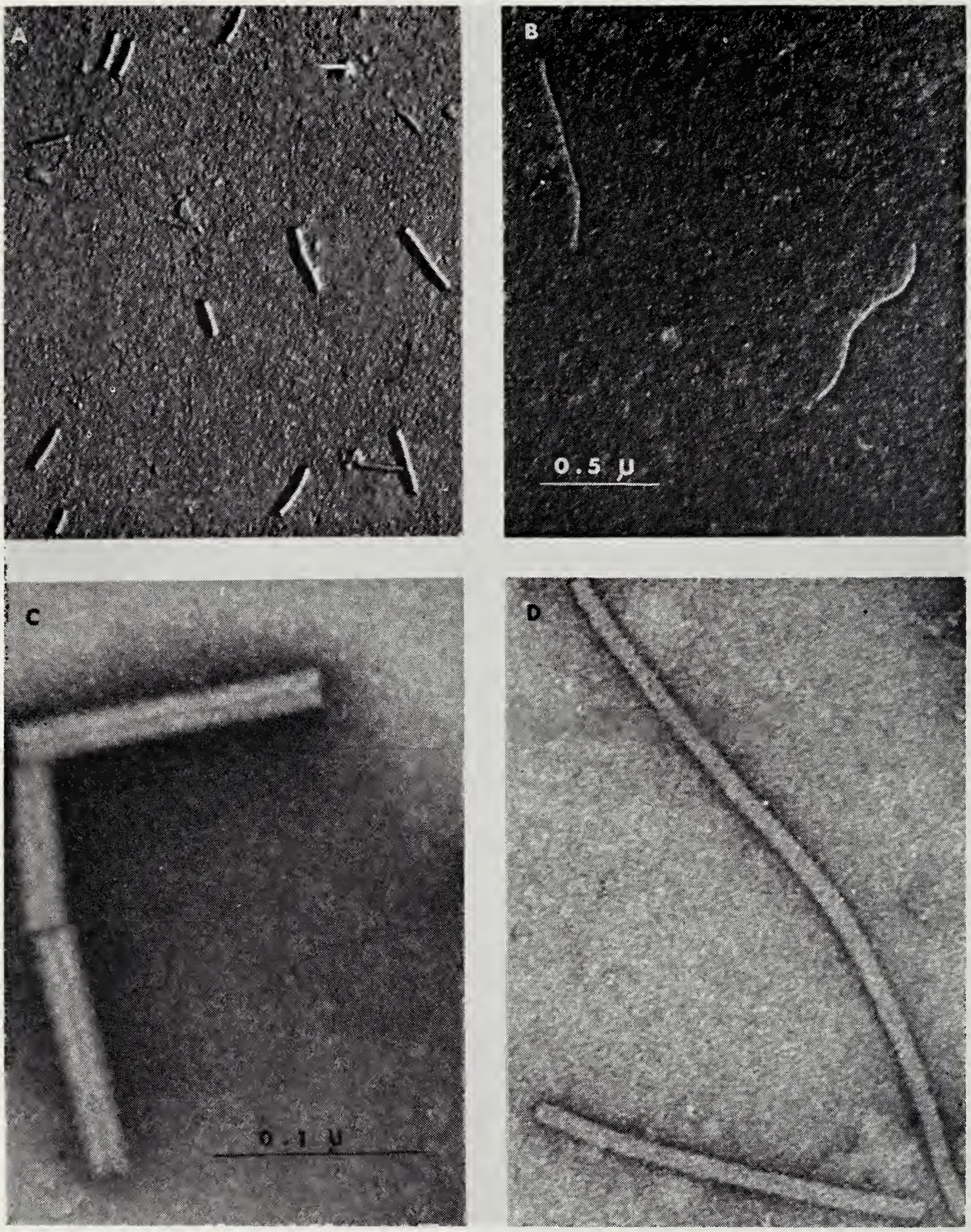

FIGURE 2. — Electron micrographs from dip preparations of leaves from viral diseased plants. A-B. Palladium shadowed mounts of particles in virus-mottled tissue. A. Leaf of wheat infected with wheat soil-borne mosaic virus. B. Leaf of corn infected with maize dwarf mosaic virus. C. Particles of wheat soil-borne mosaic virus negatively stained with phosphotungstic acid. D. Particles of maize dwarf mosaic virus negatively stained with uranyl formate. B and C. From: Brandes, J., et al., 1964.

nally, viral concentration in the freshly excised cells must be fairly high to obtain a satisfactory number of particles on a grid.

Advantages and limitations of the technique in the detection and study of non-viral ultramicroparticulates will become evident by careful use of the procedure.

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NOTE ON THE SELECTIVITY OF METHYL GREEN-PYRONIN B FOR NUCLEIC ACID

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ABSTRACT. — The selectivity of methyl green and pyronin B for DNA and RNA was examined in root tip cells under a variety of conditions which affected the staining reaction. Results indicated that: 1) Used singly, neither methyl green nor pyronin B could distinguish between DNA- and RNA-containing components in the cell, nor could they distinguish between isolated DNA and RNA even though the DNA was highly polymerized. 2) When each of the single stains was applied sequentially, methyl green could displace pyronin B only from the nucleus. When pyronin B staining followed methyl green, the pyronin B displaced methyl green from nuclei, nucleoli and cytoplasm. It was concluded that neither methyl green nor pyronin B has an inherent capacity to distinguish between DNA and RNA. However, methyl green is selective for highly polymerized DNA or chromatin when in a native state provided that the methyl green is combined with pyronin or if pyronin staining precedes the methyl green staining. In a mixture of the two dyes, pyronin B appears to compete with methyl green for stainable sites and appears to displace it from all cell structures except chromatin where the methyl green remains firmly bound, presumably because of sterochemical factors existing between the native DNA and the two cationic groups of the methyl green molecule.

The selectivity of methyl green, and of the methyl green-pyronin stain (Unna mixture) for nucleic acid has received much attention (Alfert, 1952; Barka and Anderson, 1963; Brachet, 1953; Chayen, 1952; Kurnick, 1947, 1950a, 1950b, 1952, 1955; Kurnick and Mirsky, 1950; Kurnick and Sandeen, 1960; Pollister and Leuchtenberger, 1949; Sibatani, 1952; Taft, 1951a, 1951b; Vercauteren, 1950). Although certain discrepancies exist in the literature concerning the degree of this selectivity, it is in general accepted that methyl green exhibits a greater affinity for "native" (highly polymerized) DNA than it does for either degraded DNA or RNA. When the stain is suitably prepared and applied, nuclei stain green or blue while cytoplasm and nucleoli stain pink. Methyl green selectively stains the nuclear DNA green while pyronin is not inherently selective for RNA and its specificity is determined by the use of ribonuclease (Brachet, 1953, 1957). An inherent specificity of methyl green for DNA may also be questionable since it can stain cytoplasm when applied without pyronin (Alfert, 1952; Kurnick, 1955; Sibatani, 1952). Likewise, pyronin can stain chromatin when methyl green is not present (Alfert, 1952; Kurnick, 1955; Sibatani, 1952).

This investigation was undertaken to clarify discrepant results of previous reports and to attempt a better understanding of the mechanism of the reaction.

MATERIAL AND METHODS

Allium sativum root tips were fixed in 18 single and complex fixatives commonly used for cytochemical and cytological investigations. The material was washed in water following fixation when necessary, dehydrated and embedded in paraffin.

Methyl green and pyronin B were obtained from the National Aniline Division of Allied Chemical and Dye Corporation. Purified DNA and yeast RNA were obtained from Schwarz BioRe-

search Inc. A sample of highly polymerized DNA was supplied courtesy of Dr. D. Pittman, Dept. of Microbiology, Southern Illinois University.

Methyl green was purified by repeated chloroform extraction to remove crystal violet. Solutions of dyes were modified from Taft (1951a). The mixture used (or each component if used as a single stain) contained 0.5% purified methyl green combined with 0.1% pyronin B. The stains were diluted with an equal amount of acetate buffer at pH 4.8, the optimal pH for best color differentiation (Pearse, 1960).

The staining method was similar to Brachet's method as found in Pearse (1960). Slides from each fixative were carried through the procedures simultaneously. Some pyronin B was lost during the recommended quick acetone dehydration, but the loss was slight and appeared consistent for all slides.

Observations were made on meristematic and dividing cells since staining of vacuolated or differentiated cells was seldom consistent within the same section.

Color variations resulting from the various experimental procedures were distinct enough so that the results could be scored subjectively by eye. These procedures are included below.

Results and Discussion

1. *Methyl green staining.* When unfixed root tips were stained in methyl green following pretreatment with 10% pectinase and maceration, nuclei were stained dark green, nucleoli green, and cytoplasm light green. In fixed material, regardless of the fixative, nuclei stained green, but the color intensity varied with the fixative from dark green to green. Nucleoli and cytoplasm varied from dark green to colorless.

2. *Pyronin B staining.* When unfixed material was stained with pyronin B, nuclei and nucleoli appeared dark pink and cytoplasm stained pink. In fixed material nuclei and nucleoli varied in coloration, depending on the fixative from dark red to pink. Cytoplasm varied from red to light pink.

Thus, used as single stains neither methyl green nor pyronin B exhibited any selectivity for either type of nucleic acid, though the intensity of the coloration was affected by the various fixatives.

3. *Combined methyl green-pyronin B staining.* In unfixed root tips, the Unna mixture stained nuclei emerald green, nucleoli dark pink, and cytoplasm pink. The color differentiation of nuclei, nucleoli and cytoplasm in unfixed root tips was better than in any of the fixed material. In fact, nuclei in all of the fixed material tended to be bluish rather than purely green.

4. *Sequential staining with methyl green-pyronin B.* Slides from each fixative were stained 10 min first in pyronin B and then stained in methyl green for 10 min. Nuclei varied in coloration from dark blue-green to green; nucleoli from red to faint pink. Cytoplasm was less clearly differentiated, but depending on the fixative, it varied from pink to "bluish-pink." All slides, however, showed color differentiation.

When the reverse sequence was used so that pyronin B followed the methyl green staining, then the nuclei, nucleoli, and cytoplasm were all stained shades of red or pink. Sections appeared similar to those stained with pyronin B alone and they showed little or no traces of methyl green.

Results from sequential staining indicated that methyl green could replace pyronin B only from nuclei, but never from the nucleoli, and pink coloration was never completely displaced from the cytoplasm. However, pyronin B could displace methyl green from nuclei, nucleoli, and cytoplasm. These results suggest that any bonds formed between methyl green and RNA may be considerably weaker than bonds formed between pyronin B and RNA. Sibatani (1952) also noted that methyl green was less firmly bound to RNA than to DNA. This was evident from the above procedures since RNA-containing structures (nucleoli and cytoplasm) which normally would have been stained pink with the Unna mixture are actually methyl green stainable although the green color always could be displaced by pyronin B. However, either component of the Unna mixture seemed capable of binding equally well with nuclei in the sense that either methyl green or pyronin B could displace the other from the chromatin.

5. *Model experiments with nucleic acid.* Samples of highly polymerized DNA were dissolved in M-NaCl, the stains added, and then the DNA was precipitated with ethanol and washed on filter paper. The DNA stained *pink* with pyronin B, *green* with methyl green, and *green* with the Unna mixture. After boiling the DNA for 10 min and then

staining with the Unna mixture, the DNA stained *blue* rather than green. This heat effect has been attributed to depolymerization of the DNA (Pollister and Leuchtenberger, 1949). Pyronin B staining evidently increases to produce the blue coloration. The boiled sample of DNA bound both components of the stain to some extent. Thus, the methyl green in the Unna mixture was unable to differentiate the DNA to the same extent as seen in unboiled DNA.

Commercial degraded DNA and RNA were also used. This DNA and RNA stained *dark pink* with pyronin B, *green* with methyl green, and *dark blue* or *purple* with the Unna mixture. The Unna mixture could not distinguish between these nucleic acids as it did with highly polymerized DNA. Absolutely no selectivity was in evidence, but a purple coloration resulted after staining with the Unna mixture, which is the color of the Unna mixture itself.

The results of this and the preceding procedures support the view that methyl green and pyronin B combined in the Unna mixture may compete for stainable sites in the nucleic acid. Michaelis (1947) stressed the competition that exists between cations for adsorption by the negatively charged nucleic acid phosphoric groups. Methyl green overcomes the competition offered by pyronin B only in the chromatin, i.e. highly polymerized DNA. As the DNA becomes depolymerized by heat, or fixatives (Kurnick, 1950a) so that it is no longer in its "native" state, methyl green seems progressively less able to compete with pyronin B. Therefore, as the DNA becomes more and more degraded, pyronin B uptake tends to increase in the nucleus. With degradation or depolymerization, it is assumed that the repeating distances which exist between the stainable groups in DNA are changed from their native orientation so that the two cationic groups of the methyl green molecule (see Conn, 1961 for structures) no longer "fit" the substrate (Pollister and Leuchtenberger, 1949; Vercauteren, 1950). Our experiments support the assumption that pyronin B can be more tightly bound to a substrate than methyl green unless both cationic groups of the methyl green molecule are saturated. Thus, methyl green might be displaced easily from RNA by pyronin B because of this supposedly weaker bonding as indicated from the sequential staining experiments.

The high concentration of methyl green in the Unna mixture compared to pyronin probably also influences its selectivity (Kurnick, 1955; Sabatani, 1952). This factor combined with the stereochemical relationships which presumably exist between "native" DNA and the methyl green molecule may enable the methyl green to overcome the competition offered by pyronin B for stainable sites in the chromatin. If no stereochemical factors are present in the substrate to influence the staining then the randomly arranged PO_4 groups might be able to saturate both cationic groups of methyl green as well as those of the pyronin B molecules. This could result in the purple coloration which was observed in the stained degraded DNA in the model experiments.

6. *Cold $HClO_4$ and H_2SO_4 pretreatment.* When sections were treated 15 hr with cold 10% perchloric acid (Erickson *et al.*, 1949), the basophilia, presumably due to RNA, was completely removed from cytoplasm and nucleoli. The Unna mixture stained nuclei light violet rather than green. Feulgen staining also produced a light violet nuclear coloration in unhydrolyzed sections, indicating that a slight hydrolysis was probably caused by the procedure. However, methyl green alone still stained nuclei light green, and pyronin B used alone still stained nuclei pink. The slight hydrolysis appeared to produce staining effects similar to those caused by boiling in that pyronin uptake increased and methyl green staining was reduced. Although the DNA was methyl green stainable, this stainability could not be expressed selectively by methyl green when present in the Unna mixture.

Slides were also immersed in 0.5N-H_2SO_4 at 5° C for 6 hr to remove histone (Leslie, 1961). After this treatment there was an evident increased uptake of each of the stains when used separately. Presumably this increase resulted from an unmasking of stainable groups on the nucleic acid caused by a loss of the competing histone protein. However, the Unna mixture was less able to differentiate between the nucleic acids probably because of a slight hydrolysis which resulted from this procedure as it did after the cold perchloric acid pretreatment.

Other experiments are not reported here since the results are in agreement with those adequately described in the literature. Our results essentially agree

with most reports in that methyl green appears selective for "native" or polymerized DNA (Barka and Anderson, 1963; Chayen, 1952; Kurnick, 1947, 1950a, 1950b, 1952; Kurnick and Mirsky, 1950; Kurnick and Sandeen, 1960; Pollister and Leuchtenberger, 1949; Sibatani, 1952; Vercauteren, 1950). However, for this selectivity to be realized in the cell, the methyl green must be combined with pyronin B, a fact previously noted by Sibatani (1952) or, less effectively, the pyronin B staining must precede methyl green staining. Both methyl green and pyronin B can stain nuclei, nucleoli, and cytoplasm. Likewise, each may stain both DNA and RNA. Used alone, neither dye appears to have an inherent capacity to distinguish between DNA and RNA. In a suitably prepared mixture of the dyes, pyronin B appears to compete with methyl green for stainable sites and appears capable of displacing it from all cell structures except chromatin where the methyl green remains firmly bound, presumably because of stereochemical factors existing between the native DNA and the two cationic groups of the methyl green molecule.

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Condensed from a thesis submitted to the Graduate School of Southern Illinois University in partial fulfillment of the requirements for the degree of Master of Science. Present address of senior author: Biology Department, Brookhaven National Laboratory, Upton, New York 11973.

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DAYTIME LOCATIONS AND BEDSITES OF WHITE-TAILED DEER FAWNS

CHARLES G. KJOS and G. G. MONTGOMERY
Illinois Natural History Survey, Urbana

ABSTRACT. — Locations, including bedsites, of two male white-tailed deer fawns (*Odocoileus virginianus*) were determined by radio-tracking and visual observation. Locations were restricted to areas of 2.5 acres or less. Bedsite temperatures averaged 3° F cooler than temperatures in direct sunlight near the beds. Dense overhead cover was found at 29.4 percent of the bedsites. Dense ground cover provided effective concealment from the side at 94.1 percent of the bedsites.

This paper reports daytime locations and bedsites of a tame and a wild white-tailed deer fawn on the Dixon Springs Agricultural Research Center, Pope County, southern Illinois, as determined by radio-tracking and visual sighting, June 30 through August 8, 1964.

METHODS AND MATERIALS

Both male fawns were captured by field crews at the Research Center. One fawn, captured June 13 when approximately 2 days old, was abandoned by his mother and was cared for at the junior author's home from June 18 through July 4 (Montgomery, 1968). On July 5 the fawn was released and was fed a can of warmed condensed milk two or three times daily at the release site (Fig. 1), approximately 5 miles from the capture site. This fawn is considered tame. The second fawn was about 7 days old when captured, radio-tagged and released June 2 at the release site shown in Figure 2. This fawn was not seen with his mother during the following month, but because it is unlikely that he could have survived without milk (Short, 1964, p. 457) it is assumed that he was fed by the doe.

The fawns were each radio-tagged with a miniature radio transmitter (Cochran, unpublished) held by an expandable nylon collar. A directional radio receiver (Cochran and Nelson, 1963) was used to locate the fawns.

If a fawn was not bedded when seen, only his location was recorded. If the fawn was bedded, the following additional information was recorded: temperature, 6 inches above ground level, in the bed, in shade near the bed, and in the open (direct sunlight) near the bed;

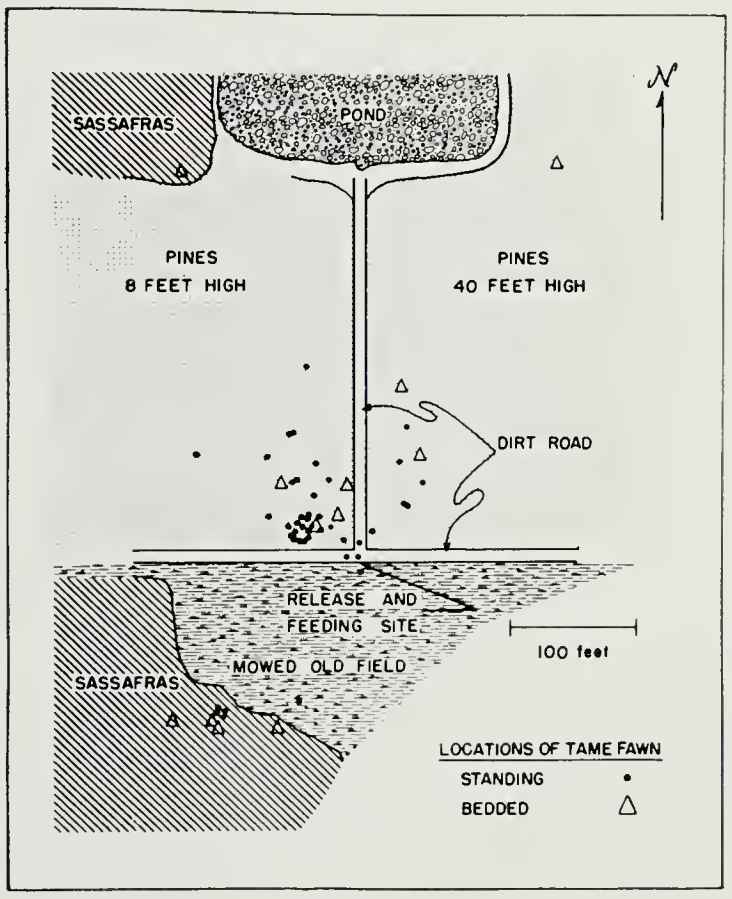

FIGURE 1. — Daytime locations and bedsites of the tame fawn July 5 through August 8, 1964. The fawn was approximately 3 weeks old on July 5.

approximate mean height, and a subjective estimate of the density of plants, if any, directly over the bed (overhead cover) and plants surrounding the bed (ground cover).

Results

The tame fawn restricted his movements to an area of about 2.0 acres (Fig. 1). A stand of loblolly pines (*Pinus taeda*) and shortleaf pines (*P.* echinata) about 8 feet tall, planted 6 feet apart, grew in the area where he was most frequently located. He was bedded 11 of the 60 times he was located (Fig. 1). The locations farthest from the fawn's release site were two bedsites at distances of about 330 and 340 feet NNW and NNE, respectively. All other locations were within 200 feet of the release site.

Overhead cover was absent from 5 of the 11 bedsites, was moderate over 4, and was dense over 2 bedsites. Ground cover, comprising the plants in the bed and those immediately surrounding it, was dense at all bedsites and averaged 32 inches in height.

The wild fawn was bedded 6 of the 33 times he was located (Fig. 2). Thirty of the locations were in or near an old-field approximately 500 feet from the relase site; only one location was less than 200 feet from the release site. The wild fawn's bedsites were similar to those of the tame fawn's in that they were distributed over much of the approximately 2.5-acre area to which he confined his movements, and apparently no bedsite was used more than once. Overhead cover was absent from two of his six bedsites, moderately dense over one, and dense over the remaining three. The average height of the vegetation immediately surrounding the bedsites was 12 inches; this ground cover was dense around all six of the beds, providing effective concealment from the side.

Temperatures in the beds of both fawns averaged 3° F cooler than temperatures in the open (direct sunlight, when present, near the beds) (Table 1), but averaged about 4° F cooler when temperatures in the open were higher than 75° F. Since shade temperatures near the bedsites also averaged about 4° F cooler than temperatures in the open, it appears that the fawns selected bedsites where they could be coolest.

Daily feedings at the same location may have tended to restrict the movements of the tame fawn. Likewise, feedings by his mother at approximately the same place each time might have limited the wild fawn's

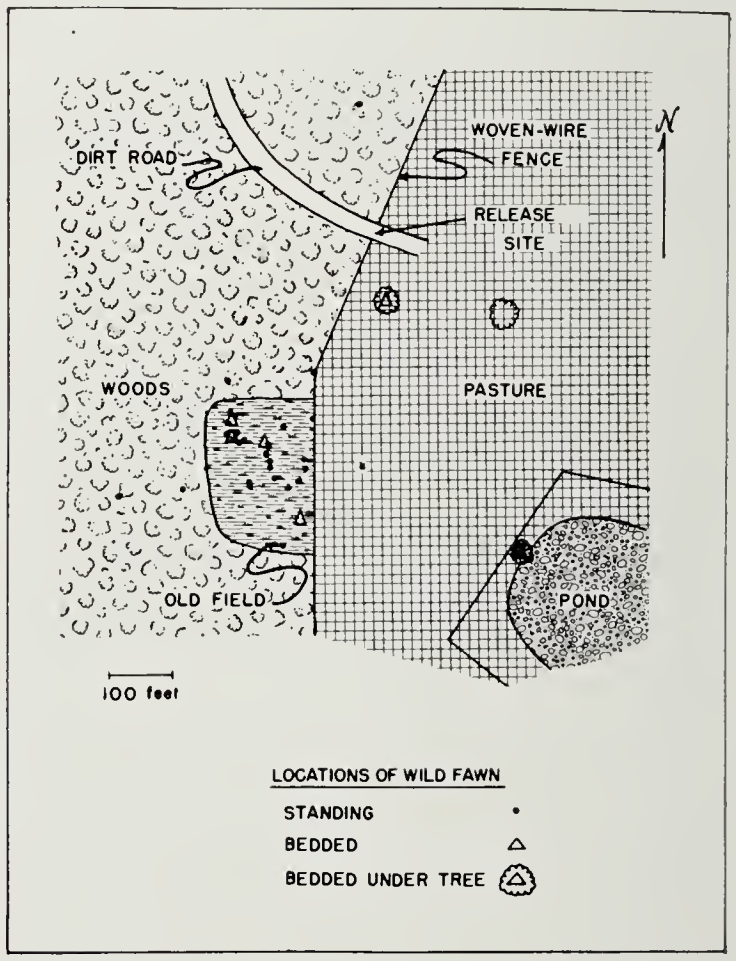

FIGURE 2. — Daytime locations and bedsites of the wild fawn June 30 through July 30, 1964. The fawn was approximately 2 weeks old on June 30.

TABLE 1.—Temperatures Associated with 11 Daytime Bedsites Used by Two Fawns In Southern Illinois, June 30 through August 8, 1964.

Time Fawn Was Seen in Bed (CST)	Cloud Cover	Temperature In Open (°F)	Temperature In Shade (°F)	Temperature In Bed (°F)	Temperature in Sunlight Minus Temperature in Bed (°F)
Tame Fawn					
0930	Hazy	93	88	90	3
1245	Absent	93	91	91	2
0900	Absent	94	87	93	1
0700	Absent	71	67	71	0
0645	Absent	75	69	69	6
1245	Absent	96	93	93	3
1830	Partial	83	80	80	3
1245	Partial	96	90	90	6
Wild Fawn					
1130	Partial	70	70	70	0
0715	Partial	86	81	81	5
1115	Partial	86	81	81	5
Average Temperatures		86	82	83	3

movements. When the observer approached, the tame fawn would bound up to be fed, whereas the wild fawn generally ignored the observer and allowed frequent approaches within 20 to 30 feet, on several occasions less than 15 feet. Although aware of the observer, the wild fawn usually continued to feed.

This study was supported by Public Health Service grant #CC-00052, from the National Communicable Disease Center, Atlanta, Georgia. Dr. Glen C. Sanderson was Principal Investigator.

ACKNOWLEDGMENTS

Dr. R. A. Evers assisted in plant identification. W. W. Cochran designed the transmitters and receiver and assisted in their use. Mrs. Judith A. Montgomery made the expandable collars to hold the transmitters, and cared for the tame fawn before his release. P. R. Robinson assisted with the field work. Dr. R. F. Labisky and Mrs. Helen C. Schultz gave editorial assistance. Present address of Montgomery: James Ford Bell Museum of Natural History, University of Minnesota, Minneapolis, Minn. 65455.

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A COMPUTER PROGRAM FOR VISCOSITY MEASUREMENTS

JERRY D. DRENNAN and BORIS MUSULIN
Southern Illinois University, Carbondale, Illinois

ABSTRACT. — A computer program is presented for the calculation of absolute viscosity based upon a capillary-tube method. The program automatically tests for erroneous data and calculates pertinent errors. The program contains a kinetic energy correction option.

This program is another in the series of programs developed for use in undergraduate research [Drennan and Musulin (1967)]. Like its predecessor, the program is directly applicable to undergraduate physical chemistry laboratory and to freshman and secondary school honors programs.

DESCRIPTION OF THE PROGRAM

The program is written in FORTRAN II (IBM, 1962) for the IBM 1620 computer with 40K storage. A monitor IBM PR 025 was used.

This program was designed for use in conjunction with the Cannon-Fenske viscometer [Weissberger, (1959), Chapter XII] in which the experimenter records flow time of a liquid dropping through a fixed capillary distance. The program calculates the average flow time for a set of time readings, the average deviation from the mean flow time, the mean error of a time reading, and the probable error of a time reading. The program rejects all readings which are greater than four times the average deviation from the mean [Pierce and Haenisch (1948), pp. 52-3]. This criterion is a modification of the statistically rigorous Chauvenet's Criterion [Worthing and Geffner (1943), pp. 170-1].

The typical experiment [Daniels, et. al. (1962)] obtains the absolute viscosity by comparing flow times of the substance to that of a reference liquid, usually water. If flow times are fast, then it is necessary to fit kinematic viscosity as a function of time to a two-constant equation through the use of two reference liquids. The present program allows the experimenter an option of using one or two reference liquids. In addition to the flow times, the viscosity and density of the reference liquid(s) are used as input information. In the case of kinetic energy correction, the program calculates probable errors for each constant in the equation, assuming only experimental error in the flow times of the reference liquids.

The program output also presents the absolute viscosity with its probable error and the fluidity with its probable error. Each output card contains the temperature of measurement and an identification number. The latter indicates whether the card contains time readings, statistical errors, viscosity, or fluidity as well as any information concerning the liquid incorporated by the experimenter.

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APPENDIX

```
C  Calculation of viscosity, fluidities, and errors
C  Monitor cards
3400032007013600032007024902402511963611300102
ZZJOB 5
ZZFORX5400
*FANDK2008
C  Reservation of storage for variables, provides for fifty time readings in one
C  set
        Dimension TR(50), TDL(50), P(50), PS(50)
C  Designation of input variables as numbers with five decimal places
     29 Format (3F10.5)
C  Two input cards with information (viscosity, density, temperature of measure-
C  ment) regarding the two standard liquids. If no kinetic correction is em-
C  ployed, the second reference card is to contain zeroes.
      1 Read 29, VHOH, DHOH, TEMP
        Read 29, VCCL, DCCL, TEMP
C  Calculation of kinematic viscosity of the two references
        VKH = VHOH/DHOH
        VKC = VCCL/DCCL
     30 Format (F10.5, I4)
C  Input card with number of readings in a set
      7 Read 2, N
      2 Format (I3)
C  Initializations
        LQ=0
        LR=0
        LP=0
        TTR=0
        KQ=0
        L=0
        AT=0
        LA=0
C  The density of the substance whose viscosity is being determined, the probable
C  error of the density, and an identification number are read in. The experi-
C  mental quantities each contain five decimal places.
        Read 30, DSUB, SGPE, KQQ
C  Calculation of the specific gravity of the substance
        SPGR = DSUB/DHOH
C  The set of time readings for the substance is read in. Provision is made for
C  specification of two decimal places. The sum of the time readings is calcu-
C  lated.
        DO 3 I=1,N
        Read 4, TR(I), JQ
C  For first reference JQ = 01, for second reference JQ = 30
      4 Format (F6.2, I3)
        L=L+1
        TDL(L)=TR(I)
      3 TTR=TTR+TR(I)
```

```
C  Calculation of the average time reading
           AL=N
           ATR=TTR/AL
C  Calculation of the sum and the sum of squares of the deviations of the time
C  readings from the average time reading
       6   PP=0
           SS=0
           DO 10 K=1,N
           IF(TDL(K)) 11, 12, 11
      12   P(K)=0
           Go to 10
      11   P(K)=ABSF(ATR—TDL(K))
           PS(K)=P(K)**2
           SS=SS+PS(K)
      10   PP=PP+P(K)
C  Calculation of the average deviation
           DV=PP/AL
C  Discard of poor time readings by Chauvenet's criterion
           FDV=4.0*DV
C  Recalculation of the average time and the average deviation after discard
           DO 13 J=1,N
           IF(FDV—P(J)) 14, 15, 15
      14   Punch 4, TDL(J), JQ
           TDL(J)=0.0
           LA=LA+1
      15   AT=AT+TDL(J)
      13   Continue
           BL=N—LA
           BTR=AT/BL
C  Branch to proceed or repeat discard process
           IF(ATR—BTR) 16, 17, 16
      16   KQ=KQ+1
C  Punch (if discard occurred) answer card with average time reading before dis-
C  card, average time reading after discard, average deviation before discard,
C  temperature of measurement, and identification number
           Punch 51, ATR, BTR, DV, TEMP,KQ
      51   Format (4F10.5, 5X, I3)
           ATR=BTR
           AT=0
           AL=BL
           Go to 6
C  Calculation of mean error of time readings
      17   AME=SQRTF(SS/(BL*(BL—1.)))
C  Calculation of probable error of time readings
           APE=.6745*AME
C  Branch according to whether the time readings were for the first reference or
C  for another substance
           IF (JQ — 1) 24, 23, 24
C  Punch (if time readings were for the first reference) answer card with average
C  time reading, average deviation, mean error of time readings, probable error
C  of time readings, temperature of measurement, and identification number
      23   Punch 25, ATR, DV, AME, APE, TEMP, JQ
      25   Format (5F9.5, I3)
C  Reservation of data of first reference liquid
           ATRH=ATR
           APEH=APE
           Go to 7
C  Branch statement (using sense switch on computer console) according to whether
C  the experimenter wishes to use a kinetic correction (switch on) or not (switch
C  off).
      24   If (sense switch 1) 26, 22
```

```
C  Branch statement (within calculation of kinetic correction) according to
C  whether the time readings were for the second reference or for another sub-
C  stance
     26  IF (JQ — 30) 21, 18, 21
C  Punch (if the time readings were for the second reference) answer card with
C  average time reading, average deviation, mean error of time readings, probable
C  error of time readings, temperature of measurement, and identification number
     18  Punch 25, ATR, DV, AME, APE, TEMP, JQ
C  Reservation of data of second reference liquid
         ATRC = ATR
         APEC = APE
C  Calculation of constants in kinetic correction equation
         ACN = (VKH*ATRH) — (VKC*ATRC)
         ACD = (ATRH*ATRH) — (ATRC*ATRC)
         AC = ACN/ACD
         BCN1 = (VKH*ATRC) — (VKC*ATRH)
         BCN2 = ATRC*ATRH
         BC = (BCN1*BCN2)/ACD
C  Calculation of probable error of each constant in the kinetic correction equa-
C  tion
         PEI = SQRTF((VKH*APEH)**2 + (VKC*APEC)**2)
         PE2 = SQRTF(2.*((ATRH*APEH)**2 + (ATRC*APEC)**2))
         PA=SQRTF(((ACN*PE2)/ACD)**2) + (PE1**2))/ACD
         PE3 = SQRTF((VKC*APEH)**2 + (VKH*APEC)**2)
         PE4 = SQRTF((ATRC*APEH)**2 + (ATRH*APEC)**2)
         PE5 = SQRTF((BCN1*PE4)**2 + (BCN2*PE3)**2)
         PB = SQRTF(((BCN1*BCN2*PE2)/ACD)**2) + (PE5**2))/ACD
         Go to 7
C  Calculation of kinematic viscosity of non-reference substance
     21  VKS = (AC*ATR) — (BC/ATR)
         V = VKS*DSUB
C  Calculation of probable error of absolute viscosity
         PE6 = SQRTF((AC*APE)**2 + (ATR*PA)**2)
         PE7 = SQRTF(((BC*APE)/ATR)**2) + (PB**2)) /ATR
         VPE = SQRTF((PE6**2) + (PE7**2))
         Go to 19
C  Calculation of absolute viscosity (with no kinetic correction) of non-refer-
C  ence substance
     22  V=VHOH*SPGR*ATR/ATRH
C  Calculation of probable error of absolute viscosity
         PEX=SQRTF((SPGR*APE)**2+(ATR*SGPE)**2)
           VPE=(SQRTF((SPGR*ATR*APEH/ATRH)**2+PEX**2))/ATRH
C  Calculation of fluidity and its probable error
     19  FL = 1./V
         FLPE=VPE/V**2
     20  Format (3F10.5, 14X, I4)
C  Punch answer card with absolute viscosity, probable error of absolute viscos-
C  ity, temperature of measurement, and identification number
         Punch 20, V, VPE, TEMP, KQQ
         LQ=KQQ+100
C  Punch answer card with average time reading, average deviation, temperature of
C  measurement, and identification number
         Punch 20, ATR, DV, TEMP, LQ
         LP=KQQ+200
C  Punch answer card with mean error of time reading, probable error of time
C  reading, temperature of measurement, and identification number
         Punch 20, AME, APE, TEMP, LP
         LR=KQQ+300
C  Punch answer card with fluidity, probable error of fluidity, temperature of
C  measurement, and identification number
         Punch 20, FL, FLPE, TEMP, LR
C  Return for next set of time readings
         Go to 7
         End
```

RESIDUAL SOIL FERTILITY AND GROWTH OF PLANTED COTTONWOOD ON UPLAND SOILS IN SOUTHERN ILLINOIS

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ABSTRACT. — Cottonwood was planted on a recently abandoned agronomic experimental field that had been used for 50 years to test crop rotations with soil and fertilizer practices. After four growing seasons, tree survival was not affected by past soil treatments. Cottonwood growth was best on plots that had received lime, but the addition of rock phosphate to limed plots depressed height growth. The effects of past fertilizer practices upon the chemical composition of the soil are given.

Each year in southern Illinois numerous upland areas that contain soils with dense subsoils are retired from agronomic production. With a greater demand for cottonwood (*Populus deltoides* Bartr.) as a pulp species, there is a need to know the productive capacity of these poorly drained upland soils for this species. An opportunity was afforded on the Raleigh Soil Experiment Field located in Saline County to test the effect of such soil conditions as well as the effect of residual soil fertility on the survival and growth of cottonwood. This experimental field was established by the Department of Agronomy in 1910 as part of their statewide program of research in crops and soils. Agronomic work was discontinued on the tract in 1962 and cottonwood was planted in 1964.

SOIL DESCRIPTION

The field ranges from nearly level to slopes up to 4 percent, with the soil types being Hoyleton and Chauncey silt loams. Where the slope is greater than 2 percent, the soil has eroded.

Hoyleton is a moderately dark-colored, imperfectly drained Brunizem soil, intergrading toward a Planosol that occurs on the slopes. It developed in 20 to 30 inches of loess over weathered glacial drift, with the native vegetation being prairie grasses. Permeability is slow because of its somewhat dense, fine-textured subsoil. Available moisture capacity is high, but the natural fertility is low. The B horizon is at a depth of less than 20 inches.

The Chauncey soil occurs on the level areas and is a moderately deep, dark-colored Planosol. Except for these features, it is similar to the Hoyleton soil in other respects.

ROTATION AND SOIL TREATMENTS

The field arrangement in 1910 included 10 plots, each one-fifth acre in size. Three plots were operated as a livestock system, making use of barnyard manure. The other seven plots represented a grain system of farming. They received no manure, but crop residues were returned to the land except on the check plots. A four-year rotation of corn, oats, mixed hay, and wheat with catch crops was used during the entire time agronomic research was in progress.

In 1924, the plots were divided and additional phosphorus was added to the east halves. A second division was made in 1952 when potassium was applied to the north halves of all plots.

The following treatments were applied singly and in combination to the west

20 plots, each one-twentieth acre in size, that were used in the cottonwood study:

O = no treatment
M = manure — 1 ton of manure applied, preceding the corn crop, for each ton of crops grown during the rotation
R = crop residues — stalks, chaff, and straw produced in rotation
L = limestone — a total of 16 tons per acre applied in seven applications from 1910 to 1924
P = rock phosphate — a total of 16 tons per acre applied in five applications from 1910 to 1924
K = muriate of potash (0-0-60) — 332 pounds per acre each rotation, 166 pounds on wheat, 166 pounds on oats
K (plus) = muriate of potash (0-0-60) — 167 pounds per acre applied biannually ahead of wheat and oats from 1952 to 1962

These basic treatments were systematically assigned in the following combinations to 10 plots: O, M, ML, MLP, O, R, RL, RLP, RLPK, and O. The other 10 plots were assigned the same treatments as the first 10 plots except for the addition of K (plus) to each plot. Treatments were applied through the 1962 growing season, when agronomic research was discontinued.

Methods

One-year-old cottonwood seedlings from a local seed source were used in the study. Seedlings were planted in the spring of 1964 in 6-inch auger holes, 20 inches deep at a 11 x 11-foot spacing. Before being planted, all lateral roots were clipped to within one-half inch of the seedlings' tap root. After planting, the seedlings were cut off at ground level. Three annual diskings for weed and grass control were required during the four growing seasons.

A composite soil sample from the 0-12 inch layer from each plot was taken from six locations per plot. Chemical analyses of the soil included readily available (.002 N sulfuric acid) P as described by Troug (1930); exchangeable K with a flame photometer after leaching with ammonium acetate; calcium and magnesium by EDTA on the evaporated ammonium acetate leachate (Malmstadt and Hadjiioannou, 1959); total N by the semi-micro Kjeldahl method (Bremner, 1960); and pH with a glass electrode.

Differences in heights between those plots receiving additional potassium and those not receiving the added potassium were analyzed by the "t" test, while all other treatments were analyzed by Duncan's multiple-range test (Steel and Torrie, 1960). Simple and multiple correlations between fertility levels and heights were determined by regression analyses. Survival was not statistically tested.

Results and Discussion

Growth of cottonwood in this study is less than that reported for this area (Minckler and Woerheide, 1968), but it must be noted that in this study we are dealing with areas of low fertility. Even the high fertility areas cannot be considered high when compared to most cottonwood sites.

The additional potassium applied after 1952 produced no significant effects on seedling height, nor did it appear to influence survival. These results were not surprising, as this soil is inherently well supplied with this element. But if the other elements were raised to a higher level, a response could be expected from added potassium. Therefore, data from plots receiving additional potassium in 1952 and plots that had received none in 1952 were combined.

Survival averaged 95 percent, with a range from 91 to 99 percent and appeared to be independent of past agricultural practices (Table 1). These results were expected, as soil moisture is the factor most often associated with plant survival, and the similarity in the moisture-holding capacity within the experimental area precludes any difference in survival of the cottonwood seedlings.

The effect of past fertilization practices on the top 12 inches of the soil

TABLE 1.—Survival and Height of Cottonwood Seedlings after Four Growing Seasons.

Treatment	Survival Percent	Average height Feet[1]
O	91	10.8
M	94	12.8
ML	97	18.5
MLP	97	17.2
R	97	15.2
RL	97	18.2
RLP	99	16.5
RLPK	92	16.9

[1] O, M, and R are significantly different (5 percent level) from all other treatments; MLP, RLP, and RLPK are significantly different (5 percent level) from ML and RL.

is shown in Table 2. Plots receiving phosphorus contained nearly 20 times as much available phosphorus as those plots to which none had been added. Differences in exchangeable potassium between treatments were small, and no trend could be detected, but exchangeable calcium and magnesium were more than twice as great on plots that were limed as on unlimed plots. Total nitrogen was slightly higher on limed as on unlimed plots, but not as great as expected. The largest quantities of nitrogen were found on the plots that had received both lime and phosphorus. The pH of unlimed areas averaged 5.1 and limed areas, 6.3. But a striking feature of Table 2 is the low available phosphorus in the nonphosphorous-treated plots. Twenty pounds per acre in the top 12 inches of soil is only a fraction of that which is needed for good tree growth. On the other hand, 400 pounds per acre at a pH of 6.5 must be influencing the availability of some of the minor elements, notably iron and zinc.

Height growth after four years was greater on limed plots than on unlimed plots, but the addition of phosphorus to limed plots depressed height growth (Table 1).

A highly significant relationship (1 percent level) was found between height growth and each soil variable except phosphorus and potassium in the simple regression analyses. The best single correlation was between

TABLE 2.—Soil Nutrients and pH in Top Twelve Inches of Soil when Cottonwoods Were Planted.

Treatment	Available P	Exchangeable			Total N	pH
		K	Ca	Mg		
	Pounds per acre					
O	20	260	2,500	2,100	4,100	5.1
M	16	320	2,300	2,000	4,200	5.1
ML	28	250	4,900	4,300	4,200	6.1
MLP	424	240	7,200	5,600	4,900	6.4
R	40	300	2,500	2,200	4,500	5.1
RL	12	120	4,700	3,600	4,300	6.0
RLP	428	170	7,400	1,700	5,000	6.5
RLPK	312	290	5,300	4,200	5,000	6.3

height and pH, but the combination of nitrogen and calcium was the most potent combination in a multiple regression analysis. This combination explained 64 percent of the variation in tree growth during these first four years.

This study substantiates the results of Lunt (1947), who found that growth of hybrid poplar was increased by nitrogen and lime fertilization. But the effect of lime (calcium) on the growth of cottonwood was more than likely an indirect one in this study. Lime not only raised the pH so that a more favorable environment existed for micro-organisms, but also contributed to soil nitrogen through better alfalfa cover. Even though there are small differences in the quantity of total nitrogen between plots (Table 2), undoubtedly there must be differences between the limed and unlimed plots in the quantities of nitrogen readily available for cottonwood growth.

The depressing effects that phosphorus has in combination with lime on tree growth have been reported in other studies. For example, Boggess and Gilmore (1960) found that rock phosphate in combination with lime depressed pine growth, and Gilmore and Boggess (1963) found this to be true for hardwoods. Their studies were on an old soil experiment field in southern Illinois with similar soil treatments but different soil types. A possible explanation for this depressing effect is that phosphorus at this high pH (on limed plots) resulted in a large percentage of the iron and zinc becoming unavailable for tree growth.

Results derived from this study show that acceptable early cottonwood growth can be obtained on these shallow upland soils if an adequate soil fertility level is maintained. The best growth in this study was about 4.5 feet per year, but there is no reason to believe that growth cannot be increased with higher soil fertility levels.

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SOME PHYSICAL AND CHEMICAL PROPERTIES OF BONE FROM BOBWHITES

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ABSTRACT. — Some physical and chemical properties of humeri and tarsometatarsi were compared for bobwhites (*Colinus virginianus*) of different sex- and age-classes representing uplands and more fertile bottomlands in southern Illinois. Length, greatest diameter and least diameter of humeri and tarsometatarsi did not differ significantly according to sex, age, sex and age classes, or soil quality. Dry weights of humeri and tarsometatarsi were significantly heavier in males than females, adults than young, young males than young females and adult females than young females. Humeri of birds from the more fertile area were significantly heavier than those from the less fertile area; metatarsi did not differ significantly. Tarsometatarsi generally had greater breaking strengths than humeri. The mean breaking stress of male humeri was significantly greater than those of females; tarsometatarsi did not show significant difference. Humeri of young birds had a significantly greater breaking stress than those of adults; but, tarsometatarsi of adult birds had a significantly greater breaking stress than those of young. Sex and age class comparisons and soil quality comparisons of humeri and tarsometatarsi showed no significant variations. Humeri had a greater percentage of ash than tarsometatarsi in all instances; sex classes showed no significant variation but young birds had greater ash percentages than adults. Among females tarsometatarsi of young had a significantly greater percentage of ash than those of adults. Differences in calcium and phosphorus content of bones from the various sex, age and soil classes were not significant.

In recent years, increasing numbers of ecologists have recognized the importance of soil fertility in its effect on the distribution, abundance and welfare of wild animals. Albrecht (1946) felt that consideration of soil quality must become the basis of wildlife management. Investigations by Leopold and Dalke (1943) with turkeys, Dale (1954) with ring-necked pheasants and Crawford (1950) with rabbits have shown relationships between soil fertility and distribution, productivity and physical and chemical properties of bone.

The purpose of the present investigation was to consider some physical and chemical properties of bones as reflected by sex and age classes of bobwhites and to relate these characteristics to general soil fertility of the collection areas.

METHODS

During January to March, 1956, bobwhite quail were collected in southern Illinois by representatives of the Cooperative Wildlife Research Laboratory. Sex determinations were verified by internal examination. Age was determined by Leopold's (1939) method. Of 195 quail collected, 164 were young and 31 were adults.

Birds were collected from nine southern Illinois counties representing both glaciated and non-glaciated upland soils (Hamilton, Perry, Union, Washington, Jackson, Franklin, Marion and Williamson counties) and Ohio River bottomland soils (Massac County). According to Stefferud (1957), most of the upland soils of southern Illinois are acid and

low in organic matter and plant-food elements. The alluvial soils of the Ohio river bottoms are reasonably fertile showing approximately 4.5 times more organic matter, 3.5 times more nitrogen, 2.6 times more phosphorus, 1.4 times more potassium, 4.1 times more magnesium and 6.2 times more calcium than adjacent upland soils (Wascher et al. 1950). No specific details were available concerning soil improvement practices; however, much of the upland was reported to not have a history of fertilizer usage.

Preparation of Materials

Bones used in all analytical procedures were the left tarsometatarsus and the right humerus. These were chosen on the basis of their similar size and sufficient weight to produce adequate amounts of ash for chemical analyses. If these specific bones were not available in usable condition, their exact opposites were used.

Bones were removed from the birds, fleshed, and cleaned either by hand or dermestid beetles. After cleaning, bones were dried for 2 weeks at 40° C, to equalize moisture content, and then stored in air-tight, glass vials until used in testing procedures.

Physical Determinations

The total length, least and greatest diameters, weight, breaking stress and wall thickness were determined for each tarsometatarsus and humerus. Total length was measured to 0.10 mm with a vernier caliper. Least and greatest diameters at a point midway between the extremities were measured to 0.001 mm with a micrometer caliper. Measurement of the least and greatest diameter was necessary because the bones were not perfectly round; this necessitated the use of both measurements in subsequent calculations of breaking stress. Total weight of the bones was determined on a Roller-Smith balance (calibrated to 0.2 mg).

Breaking stress of an entire bone depends upon strength of the bony material as well as the shape and dimensions of the bone. Breaking stress was determined according to a modification of procedure described by Bell et al. (1941). It was calculated from the formula:

$$S_1 = \frac{M}{a\,b\,t}$$

where S_1 is breaking stress, M is the bending moment at the fracture, a is the greatest diameter, b is the least diameter, and t is the wall thickness at point of fracture.

Wall thickness (t) was measured to 0.1 mm, with an ocular micrometer in a dissecting microscope. Measurements were made on the broken edge of the medial wall of the bone at the point of fracture.

The bending moment (M) is greatest at the midpoint of a long bone (Bell et al. 1941). It was computed from the formula:

$$M = \tfrac{1}{4}\,WL$$

where W is the breaking force and L the length of the bone. In determination of the breaking force (W), the bone was supported at both ends by inserting the ends into depressions in two blocks of wood arranged on a track above table top. A weight pan was suspended by means of a wire hook encircling the exact middle of the bone shaft. Sand was added to a bottle in the pan until the bone broke. Weight of sand, bottle and pan was determined and used as the reading for breaking force.

Chemical Determinations

Chemical determinations were performed on 55 humeri and 61 tarsometatarsi, representing each sex, age and soil class. After breaking, the pieces of the broken bone were weighed and then ashed by a modification of a process described by Morgulis (1931). Ashing was carried out in a muffle furnace at 600° C, for 24 hr. A bone-ash solution was prepared by dissolving 250 mg of the combined ash from a tarsometatarsus and humerus of one bird in 2 ml of concentrated hydrochloric acid and diluted with water to 250 ml in a volumetric flask. Calcium, phosphorus and magnesium were quantitatively determined by analysis of aliquots of this solution.

Calcium was determined volumetrically by a modification of a process described by Furman (1939). It was precipitated from the solution as ammonium oxalate which was dissolved in sulfuric acid and titrated with 0.05 N potassium permanganate.

Phosphorus was measured colorimetrically from an aliquot of the bone-ash solution by the Fiske-Subbarow method as described by Hawk et al. (1947). A Spectronic 20 colorimeter (Bausch and Lomb) was used. In the presentation of data and analyses, all significant differences are based on a 95 percent level of confidence.

Results and Discussion

Physical Determinations

Dimensions: The humeri and tarsometatarsi of all birds had a mean length of 35.5 mm and 32.3 mm, respectively. There were no significant differences according to sex, age, sex and age classes and soil quality. The mean greatest diameters were 3.4 mm for all humeri and 2.6 mm for all tarsometatarsi. There were no significant differences according to sex, age, sex and age classes and soil quality. The mean least diameters of all humeri and tarsometatarsi were 2.6 mm and 2.0 mm, respectively. Least diameters of humeri and tarsometatarsi were not significantly different according to sex, age, and sex and age classes and soil quality.

There were no significant differences in wall thickness according to sex, age, sex and age or soil quality. Values ranged from a low of 0.37 mm for tarsometatarsi of adult males to a high of 0.43 mm for tarsometatarsi of all females.

Weight: The mean dry weight of all humeri was 286.9 mg and 191.2 mg for all tarsometatarsi (Table 1). The humeri of males were heavier than those of females (mean difference 8.1 mg) while the tarsometatarsi of males were heavier than those of females (mean difference 7.0 mg). Humeri and tarsometatarsi of adults were heavier than those of young, the mean difference being 6.3 mg for humeri and 5.6 mg for tarsometatarsi. All of these differences were significant.

Humeri and tarsometatarsi of young males were significantly heavier than those of young females; the mean difference being 9.9 mg between humeri of young males and females and 11.3 mg between tarsometatarsi of young males and females (Table 1). Among adults humeral and tarsometatarsal weights of males were not significantly heavier than those of females. In the case of females humeral and tarsometatarsal weights of young were 12.3 mg and 15.1 mg lighter, respectively, than those of adults; these differences were significant. Humeri and tarsometatari of young males were not significantly different in weight from those of adult males.

Humeri from bottomland area birds were significantly heavier than those from birds of the less fertile upland (mean difference 10.7 mg). A small numerical difference in mean weights of tarsometatarsi (Table 1) between areas of differing fertility was not statistically significant.

In Missouri femurs from rabbits collected on high fertility regions were 12% larger than those from rabbits collected on low fertility regions (Crawford 1950). The lack of correlation in our study with soil fertility may be partially due to the size of the sample or insufficient difference in fertility between upland and bottomland soils. Bell et al. (1941) found that the external dimensions of femora were not altered by varying the calcium intake in the diet of experimental rats.

Breaking Stress: Humeri had a mean breaking stress of 13.4 kg/mm^2, and tarsometatarsi, 16.0 kg/mm^2 (Table 2). The humeri of males had a mean breaking stress 3.2 kg/mm^2 greater than did females. This difference was statistically significant but that for tarsometatarsi was not. One might expect a significant difference

TABLE 1.—Dry weights of humeri and tarsometatarsi of bobwhites, southern Illinois.

	Humerus				Tarsometatarsus			
	Number specimens	Mean weight (mg)	Standard deviation	Range	Number specimens	Mean weight (mg)	Standard deviation	Range
According to Sex								
Female	68	283.0±4.1	33.8	207.8—348.0	72	189.0±3.1	26.4	134.8—316.8
Male	63	291.1±3.5	27.5	250.6—366.8	68	196.0±3.2	26.6	152.0—316.8
Age								
Young	113	286.0±2.3	24.1	207.8—366.8	123	190.5±2.5	27.3	134.8—316.8
Adult	18	292.3±6.7	28.3	235.6—357.6	17	196.1±4.2	17.4	163.6—220.8
Sex and Age Classes								
Young								
female	59	281.3± 3.0	23.0	207.8—348.0	65	185.2±3.4	27.1	134.8—287.6
male	54	291.2± 3.8	27.7	250.6—366.8	58	196.5±3.6	27.6	152.0—316.8
Adult								
female	9	293.6±10.7	32.1	235.6—348.0	7	200.3±6.3	16.7	164.0—220.8
male	9	290.6± 8.9	26.6	264.6—357.6	10	193.1±5.5	17.4	163.6—216.6
Site of Collection								
Massac County	16	294.1±8.6	34.2	244.2—366.8	21	192.2±5.7	26.2	140.4—271.6
All Others	115	283.4±2.3	24.5	207.8—365.6	119	190.8±2.4	26.3	134.8—287.6
Total birds	131	286.9±2.3	26.0	207.8—366.8	140	191.2±2.2	26.3	134.8—316.8

in the breaking stress values of males and females because of the demands of egg laying. We have no valid explanation for the fact that a significant difference was found only for humeri.

Humeri of young birds had a significantly greater breaking stress (2.8 kg/mm^2) than those of adults; however, tarsometatarsi of young had a significantly lesser breaking stress (2.6 kg/mm^2) than adults (Table 2). As one would suspect a significant age variation in breaking stress, this variation seemed contradictory.

TABLE 2.—Breaking stresses of humeri and tarsometatarsi of bobwhites, southern Illinois.

	Humerus				Tarsometatarsus			
	Number specimens	Mean breaking stress (kg/mm^2)	Standard deviation	Range	Number specimens	Mean breaking stress (kg/mm^2)	Standard deviation	Range
According to Sex								
Female	21	12.2±0.68	3.1	7.2—16.5	21	15.2±0.61	2.8	10.1—20.0
Male	29	15.4±0.56	3.0	8.8—18.9	36	15.6±0.45	2.7	8.7—22.0
Age								
Young	32	15.0±0.51	2.9	7.8—18.0	37	13.7±0.48	2.9	8.7—21.6
Adult	18	12.2±0.73	3.1	7.2—18.0	20	16.3±0.56	2.5	12.4—22.0
Sex and Age Classes								
Young								
female	12	13.1±0.66	2.3	7.8—16.5	12	14.8±0.84	2.9	10.1—20.0
male	20	14.6±0.56	2.5	9.7—18.9	25	15.1±0.60	3.0	8.7—21.6
Adult								
female	9	10.8±0.93	2.8	7.2—14.5	9	15.8±0.37	1.1	12.1—17.5
male	9	13.6±1.03	3.1	8.8—18.0	11	16.7±0.90	3.0	12.4—22.0
Site of Collection								
Massac County	14	13.1±0.86	3.1	7.4—18.9	21	15.3±0.63	2.9	8.7—19.6
All others	36	13.5±0.77	4.6	7.2—18.8	36	15.6±0.45	2.7	10.1—22.0
Total birds	50	13.4±0.36	2.5	7.2—18.9	57	16.0±0.37	2.8	8.7—22.0

The various sex and age class comparisons revealed no significant difference in the mean breaking stress of either the humeri or the tarsometatarsi (Table 2). Although not statistically significant, humeri of adult females had the least breaking stress. These data agree with the data on ash percentages.

There were no significant differences between the breaking stress values of humeri or tarsometatarsi of birds from the bottomland area and those from the upland area (Table

2). Thus, the breaking stress did not vary with the fertility of the southern Illinois soil areas represented in this study. However, Crawford (1950) found that the breaking strength of bones from Missouri rabbits varied directly with the fertility of the soil from which they were collected. Bell et al. (1941) noted that when the calcium intake of rats was increased, the bending moment, a measure of the strength of the whole bone, also increased up to an intake of 36%, after which the bone did not seem stronger. The strength of the bony material, as indicated by breaking stress tests similar to the ones used in our study, was unaffected by the amount of calcium in the diet.

Percent Ash: There were no significant differences in the percentage of ash in the humeri or tarsometatarsi of males and females. But, there was a significant difference between the ash content of young and adults; humeri of the former averaged 1.4% more ash than those of the latter. The ash content of tarsometatarsi of young birds was 3.0% greater than that of adults (Table 3). These values suggest an increased utilization or decreased deposition of inorganic minerals in the bones with increasing age.

Ash content of tarsometatarsi was 4.1% higher for young than adult females (Table 3). The significant difference was expected because of the activity of estrogen in the adult females prior to the egg-laying period. Calcium is mobilized by estrogen from the skeleton and held in the bloodstream for eggshell formation (Nalbandov 1948).

No other significant sex or age difference in the ash content of the bones was found according to sex and age. There were no significant differences in the humeral or tarsometatarsal ash content of birds from bottomland when compared with those of the upland.

Calcium: Comparison of the mean percent calcium of bones representing the various sex, age, sex and age and soil classes revealed no significant variations. The mean percent calcium was approximately 38.0, ranging from 35.2% to 41.1%.

Phosphorus: The percent phosphorus in bones did not differ significantly according to sex, age, sex and age or soil quality. The mean percent phosphorus was approximately 18.6 ranging from 15.8% to 20.0%.

Acknowledgments

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TABLE 3.—Percentage of ash in humeri and tarsometatarsi of bobwhites, southern Illinois.

	Humerus				Tarsometatarsus			
	Number specimens	Mean percent ash	Standard deviation	Range	Number specimens	Mean percent ash	Standard deviation	Range
According to Sex								
Female	24	65.2±0.37	1.8	62.4—70.0	24	55.5±0.72	3.5	41.7—64.1
Male	31	66.5±0.56	3.1	63.2—72.8	36	57.0±0.65	3.9	47.1—67.3
Age								
Young	35	66.4±0.32	1.9	63.2—72.8	40	57.7±0.57	3.6	41.7—67.3
Adult	20	65.0±0.38	1.7	62.4—67.8	20	54.7±0.89	4.0	46.8—61.3
Sex and Age Classes								
Young								
female	13	65.7±0.50	1.8	63.3—70.0	14	57.4±0.78	2.9	41.7—64.1
male	22	66.8±0.45	2.1	63.2—72.8	25	57.5±0.82	4.1	48.6—67.3
Adult								
female	9	64.6±1.90	5.7	62.4—67.8	11	53.3±1.30	4.3	46.8—61.3
male	11	65.5±1.17	3.9	63.2—67.6	10	55.9±1.76	3.4	47.1—59.2
Site of Collection								
Massac County	16	66.1±0.53	2.1	63.6—72.8	21	55.3±0.68	3.1	46.8—60.1
All others	39	65.8±0.29	1.8	62.4—60.2	39	57.4±0.61	3.8	41.7—64.1
Total birds	55	65.9±0.24	1.8	62.4—72.8	60	56.6±0.49	3.8	41.7—67.3

POPULATION DYNAMICS OF THE DEER HERD IN WESTERN POPE COUNTY, ILLINOIS

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ABSTRACT. — The population dynamics of the white-tailed deer (*Odocoileus virginianus*) was studied from 1965 through 1966 on a 19,840-acre area on the Shawnee National Forest including the 5,400-acre Dixon Springs Agricultural Center in western Pope County, Illinois. Population trends, based on data from compulsory check-stations during shotgun hunting seasons in both years, were indicated by: Lincoln Index calculations for radio-marked and unmarked deer, (2) age compositions among deer killed, and (3) hunter success ratios. Lincoln Index calculations indicated a substantial decrease in the population from 1965 to 1966. Combined data for the 2 years revealed that 42 percent of the deer killed on the area were fawns, 73 percent were 1½ years old or younger, and only 11 percent were over 2½ years old. Hunter success for northern Pope County was one deer per 2.9 hunters in 1965, one deer per 5.5 hunters in 1966, and one deer per 8.0 hunters in 1967.

Because many residents complained of seeing fewer deer in 1965 and 1966 than in recent years, we decided to make a quantitative investigation of the population dynamics of the white-tailed deer herd from 1965 through 1966 on a 19,840-acre area in western Pope County, Illinois. This research was in connection with a 4-year study of deer in the spread of zoonoses, which began in January, 1964, on the Shawnee National Forest (SNF), including the 5,400-acre Dixon Springs Agricultural Center (DSAC) in southern Illinois.

METHODS

Population trends were studied using the following criteria: (1) Lincoln Index (Lincoln, 1930), (2) age composition, and (3) hunter success. Deer were captured in a variety of ways (Hawkins et al., 1967*b*; Montgomery and Hawkins, 1967; Hawkins et al., 1968), radio-marked with 53-mc transmitters (unpublished data, Illinois Natural History Survey) and released at capture sites. In addition, 28 deer were translocated from other areas in southern Illinois, radio-marked, and released on the study area (Hawkins and Montgomery, 1967*a*). To aid visual observation of marked deer, females were marked with a vinyl-coated nylon collar (Hawkins et al., 1967*c*). Locations of radio-marked deer were continually monitored with a semi-automatic radio-direction-finding (RDF) system similar to the one described by Cochran et al. (1965).

Radio-marked deer were considered alive and available to be shot during a hunting season if they were being tracked by the RDF system or had been observed in the field during the month prior to the beginning of the annual shotgun hunting season. The 19,840-acre area in which the deer populations were compared for 1965 and 1966 was determined by detailed study of over 100 daily and seasonal radio-location maps (Figures 1 & 2) and over 130 observations or radio locations (Figure 3) of radio-marked deer with a hand-held receiver (Cochran and Lord, 1963; Verts, 1963). The locations were compiled by township, range, and section on a comparable basis with the kill data. Any section in which a radio-marked deer was located at any time was included to derive the

total area. Movement data were analyzed with an IBM 7094 computer; figures were plotted on a CALCOMP 564 plotter at the University of Illinois.

Each successful hunter was interviewed at a compulsory check-station to determine the township, range, and section where he had killed his deer. The results of these interviews, when applicable to the 19,840-acre area defined on the basis of locations used by radio-marked deer, were used to estimate the numbers of deer killed on the area. The age of each deer brought to the check-station was estimated on the basis of tooth emergence and wear (Severinghaus, 1949).

We assumed that there was no bias either for or against killing radio-marked deer. This was believed to be a valid assumption because it had been widely advertised before the hunting seasons began that radio-marked deer were legal game. Several of the hunters that shot radio-marked deer reported that they did not know, prior to shooting, that the deer were marked. Some hunters may have passed up shots at radio-marked deer, while some radio-marked deer may have been killed because they were more visible. At the beginning of the hunting season each year two of the radio-marked deer available to be killed were transplants and may have been more vulnerable to hunting than native deer.

The shotgun hunting season for each of the 2 years consisted of two 3-day weekends beginning on Friday morning. In 1965, the two weekends were 2 weeks apart, while in 1966 they were 3 weeks apart.

Results

Fourteen radio-marked deer were known to be alive and randomly scattered over the 19,840-acre area at the beginning of the split, 6-day, shotgun deer-hunting season which began November 19, 1965 (Table 1). Two hundred and twelve deer were killed in this area during the 1965 hunting season; seven of these were radio-marked. Thus, 50 percent of the radio-marked deer known to be alive at the beginning of the hunting season were killed. By use of simple Lincoln Index calculations, the prehunt population was determined to be 424 deer (one deer per 49 acres); since half of these were removed, the posthunt population was 212 deer. Four radio-marked deer were known to be alive on the

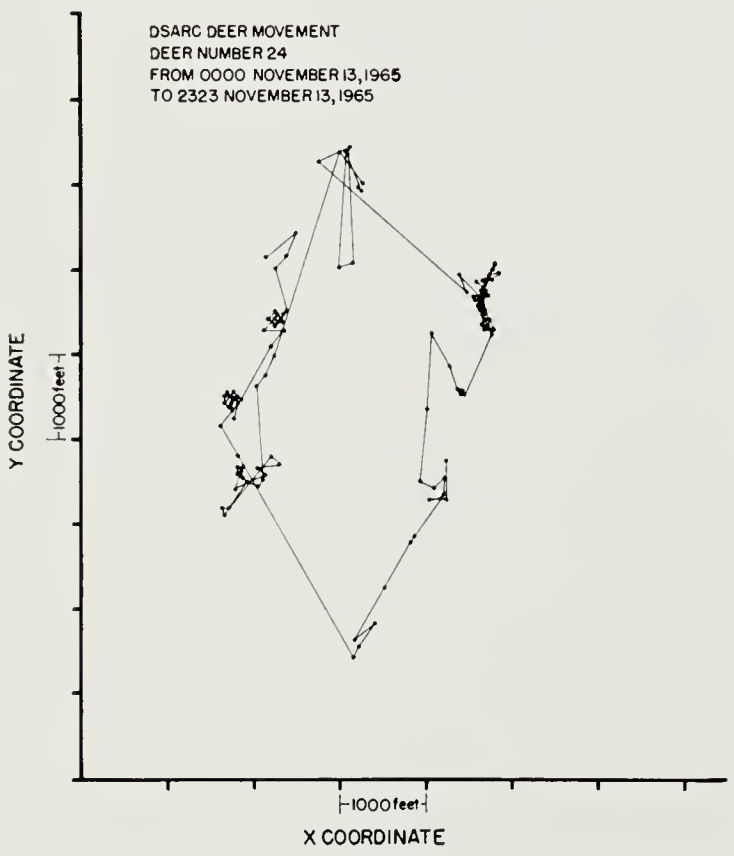

FIGURE 1. — Example of a daily range map of a radio-marked deer.

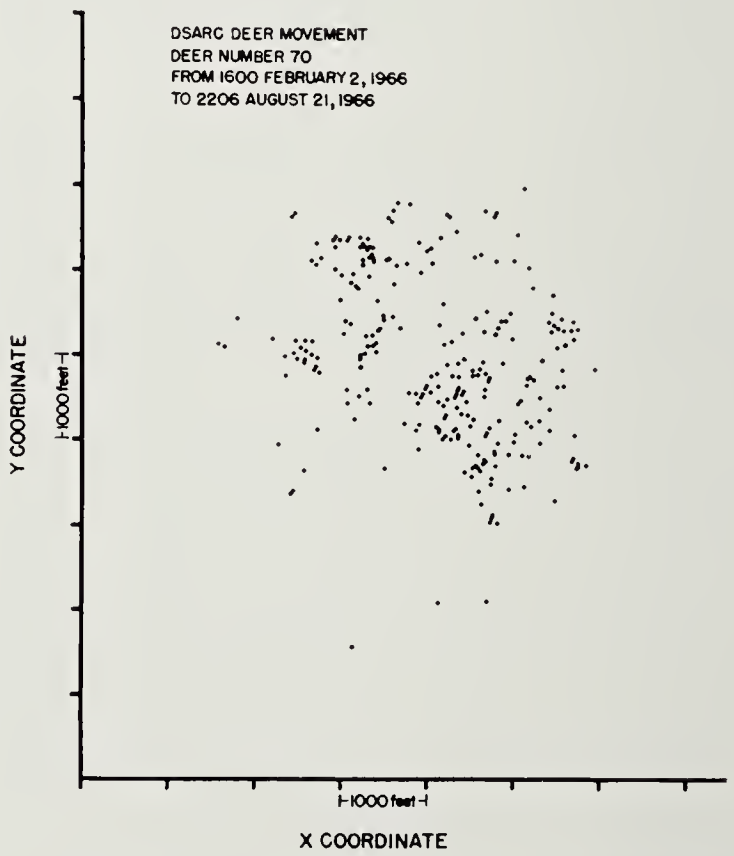

FIGURE 2. — Example of a seasonal range map of a radio-marked deer.

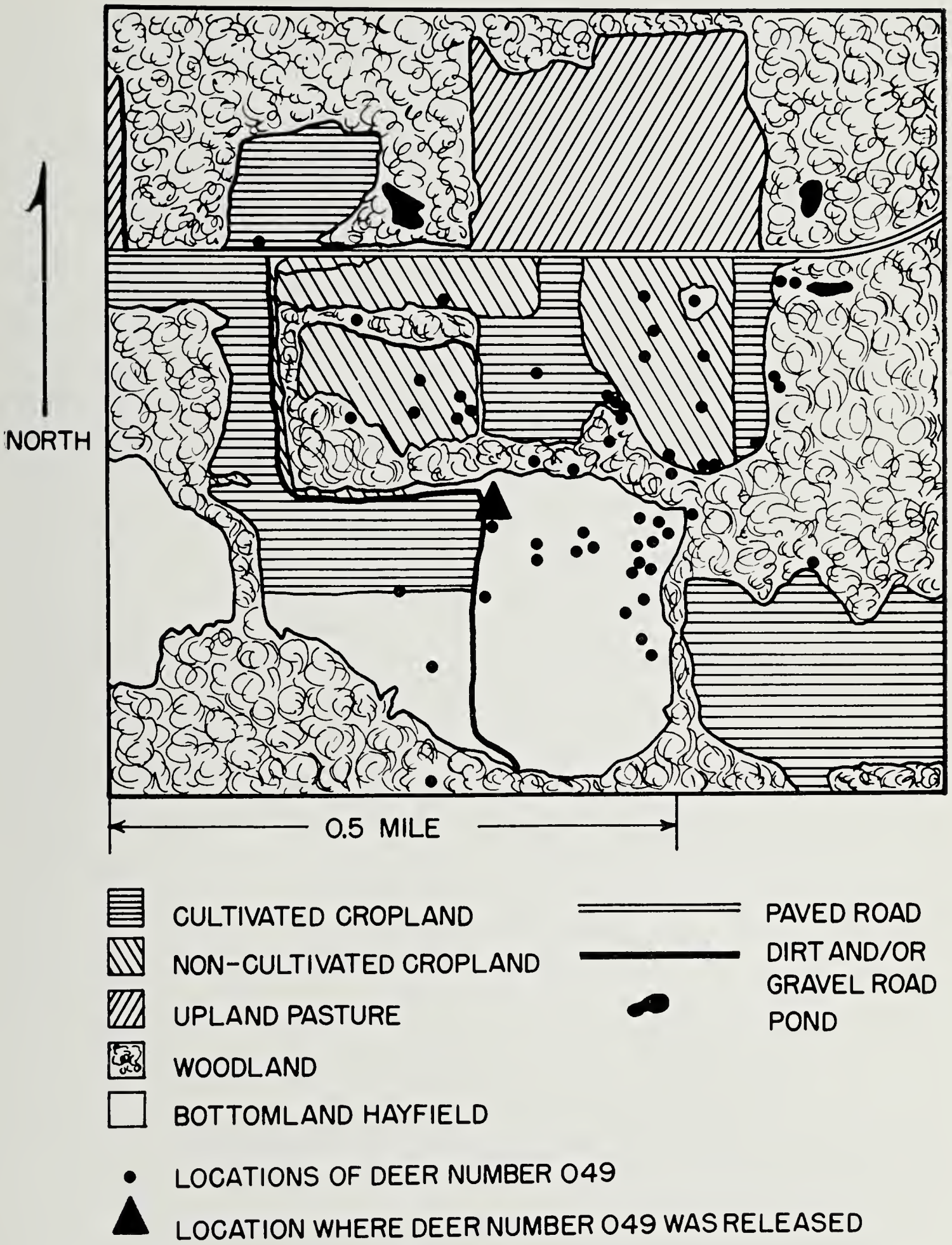

FIGURE 3. — Example of a seasonal range map plotted from visual observations after location with a hand-held receiver.

same 19,840-acre area at the beginning of the shotgun deer-hunting season in 1966 (Table 1). One hundred forty-one deer were killed in this area; two of these were radio-marked. Thus, as in the preceding year, 50 percent of the radio-marked deer known to be alive at the begin-

TABLE 1.—Radio-marked and Total Deer Killed During Shotgun Deer-Hunting Seasons on a 19,840-acre area in Western Pope County, Illinois, 1965 and 1966.

Year	Radio-marked Deer Known to be Alive at Beginning of Hunting Season	Radio-marked Deer Killed During Hunting Season	Total Deer Killed During Hunting Season
1965	14	7	212
1966	4	2	141

ning of the hunting season were killed. Lincoln Index calculations showed that the prehunt population was 282 deer (one deer per 70 acres) and the posthunt population was 141 deer. Because of the small sample of radio-marked deer each year, analyses did not reveal a significant difference in the populations; however, we believe the downward trend indicated for 1966 is valid.

There was no significant difference between 1965 and 1966 in the age composition of the deer killed on the 19,840-acre area, according to Chi-square calculations (Table 2). However, when both years were considered, 150 (42 percent) of the 353 deer killed were fawns and 257 (73 percent) were 1½ years old or younger. Conversely, only 42 (12 percent) of the deer killed were over 2½ years old. Andrews and Calhoun (1968 p. 7) found that over 40 percent of the kill in 1965 and 1966 for Pope County were fawns; Nixon (1963) concluded that harvest rates of fawns in excess of 40 percent were probably too high to maintain stability of the herd.

Severinghaus and Cheatum (1956, p. 477) stated that hunter success may give an indication of deer population trend. A total of 4,147 shotgun deer-hunting permits were issued for northern Pope County in 1965; 1,427 deer were harvested on this area for a hunter success ratio of one deer per 2.9 hunters. Likewise, 6,576 permits were issued in 1966 for the same area; 1,190 deer were harvested for a hunter success ratio of one deer per 5.5 hunters (personal communication, Mr. Thomas R. Evans, Superintendent, Division of Game, Department of Conservation).

TABLE 2.—Sex and Age Composition of the Deer Killed During Shotgun Deer-Hunting Seasons on a 19,840-acre in Western Pope County, Illinois, 1965 and 1966.

Year	Males				Females			
	Fawn	Yearlings	2½ Years	Over 2½ Years	Fawn	Yearlings	2½ Years	Over 2½ Years
1965	55	29	13	10	37	29	23	16
1966	34	31	1	3	24	18	17	13

One obvious problem of calculating hunter success is the impossibility of determining exactly how many of the hunters that obtained permits actually hunt; however, this bias probably does not vary from year to year. Weather may have affected the deer kill in 1966 and lowered the hunter success; however, there were only slight differences with respect to temperature and precipitation between the 2 years at the DSAC weather station. A 2-inch rain fell on the fourth day of the 1966 season, compared with about ½ inch of rain on the third day of the 1965 season (U. S. Dept. Commerce, Weather Bureau; 1965 and 1966).

The success ratio for 1967 in Pope County was one deer killed per eight hunters (Ill. Dept. Conserv.), which was considerably poorer than the 1966 ratio. This may have resulted, in part, from bad weather and a continuous 6-day season beginning on Friday, instead of a split season as in the past. Murphy (1966 (1965), p. 143) reported that when the 6-day deer season opened on Friday in Missouri, the season was, for all practical purposes, reduced to only 3 days.

The above information seems to indicate that there was a substantial decrease in the deer herd between 1965 and 1966 that may have begun in 1964, as suggested by Andrews and Calhoun (1968 p. 4), who have studied the population dynamics of the Pope County deer herd since the first hunting season in 1957. Although most of the herd reduction was believed to have resulted from shotgun hunting, 8 to 10 deer are killed by automobiles each year and 2 or 3 by bowhunters on the DSAC (personal communication, Mr. Glen Hombirg, Conservation Officer for Pope County). Illegal hunting may have been an important factor in the reduction of the deer herd during the period of this study; at least one radio-marked deer was illegally killed. Natural mortality, although believed to be low, has not been investigated thoroughly. Ohio suffers approximately 24 percent herd reduction each year in areas hunted (Severinghaus and Cheatum, 1956, p. 179-180). Game managers of that state realize that losses of this magnitude cannot be sustained indefinitely; therefore, they hold open season in particular areas only every 2 or 3 years (Severinghaus and Cheatum, 1956, p. 184).

We make the following recommendations with regard to management of the deer herd in northern Pope County: (1) a continuous 6-day season, as in 1967, until the herd has recuperated; (2) a reduction in the number of hunting permits issued; and (3) should these measures not restore the population to pre-1965 levels, closing the season for a year or two.

Acknowledgments

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Schultz, both of the Illinois Natural History Survey, offered editorial suggestions. P. R. Robinson, R. A. Montgomery, L. D. Martoglio, and James Seets assisted with capture of deer. The present address of R. E. Hawkins is Cooperative Wildlife Research Laboratory, Southern Illinois University, Carbondale; the present address of G. G. Montgomery is James Ford Bell Museum of Natural History, University of Minnesota, Minneapolis.

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PLANT DISTRIBUTION ON HILLS SURROUNDING KABUL, AFGHANISTAN

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ABSTRACT. — Afghanistan is an arid country with barren, rocky hills and mountains. Yet, 129 species of plants were collected from the hills surrounding Kabul. Along ten transects, between 2130 to 2310 meters in height the vegetation was examined at elevation differentials of 30 meters. Frequency and cover data were collected and Importance Values computed. Data were also taken on area of soil, solid, and broken rock and degrees of slope.

Eighty-four plant species were found with varying degrees of recurrence and 19 species were the dominants. The dominant species were well represented in eight transects. In two transects, numbers seven and eight, only 11 dominants were found. Correlations between total area of rock and of soil with cover were highly significant statistically. An effort was made to correlate plant species with elevations. Forty-six percent of the plant species were apparently not affected by elevation differences, since they were found at all elevations studied. In the lower elevational ranges to about midway, 33 percent of the species were found. Eighteen percent of the total species grew from midway to the upper elevations, with only 2 percent exclusively in the upper levels.

For the past one hundred years scientists and travellers have been making plant collections in various parts of Afghanistan. Some of the earliest were British explorer-naturalists. More recently there have been German, Russian, Austrian, French, English and American botanists in the field. In 1959, M. Pabot, a French plant ecologist, visited the country for five months and made a rapid survey of the range lands. His collection of 1,800 plants was hastily classified and deposited with the Range and Forest Division of the Afghan Ministry of Agriculture.

Most of the plant collections which have been made have been sent to various parts of the world, and are not available for study in Afghanistan. The European collections are now, for the most part, gathered in Vienna, where Dr. Karl Reichenger and his associates are working on a systematic flora of the Iranian plateau, an area which includes sections of Afghanistan. A definitive work is being organized and should be completed within the next fifteen years. The Kyoto University Scientific Expedition to the Karakoram and Hindu Kush in 1955 (Kitamura, 1955, 1964) collected plants which are now in Japan. The Pabot (1959) collection is about the only extensive collection left in Afghanistan, for which no systematic keys are available.

The writer was granted permission to see the Pabot collection for half an hour, after many requests. There was a field list of the 1,800 plants M. Pabot collected. The plants themselves were untreated, unmounted, and stored in the original newspaper

drying wrappers in which they were placed after collection. They have been placed in closets where continual rat and insect damage is rendering the collection less useful every day.

As can readily be seen, little has been done with plant studies other than collection of plants and the beginning of a systematic flora for the area. During a two-year assignment in Afghanistan a study was begun of the distribution of plants on hills surrounding Kabul.

Description of the Area

Most travellers to the rugged country of Afghanistan comment particularly on the bare hills of the country. Fraser-Tytler (1962), speaking of the mountainous aspect of this land, referred to the Hindu Kush as the entire main range of mountains which traverses the country for 1000 kilometers from east to west. The Hindu Kush properly called (Fig. 1), however, takes off in a southwesterly direction from the Sarikoh and Muztagh ranges at a point in the glacial region of the craggy Pamirs (75° E. Long.; 37° 45′ N. Lat.). It gradually diminishes in altitude from 7000 meters, until opposite Kabul it is 5000 meters. One hundred sixty-five kilometers or so to the northwest of Kabul the Koh-i-Baba range, overlapping the western extremity of the Hindu Kush, prolongs the watershed west past Bamiyan.

Only in the Kunar Valley in Nuristan to the northeast of Kabul are the deep ravines and precipitous slopes clad in magnificant forests of deodar, pine and larch. Farther west towards Kabul the ridges are bare and brown, sparsely covered in places with coarse grass which affords seasonal grazing for the flocks of sheep of the nomads. The general impression is of a wild, desolate, little-known country, a country of great peaks and deep valleys, of precipitous gorges and rushing grey-green rivers; a barren, beautiful country of intense sunlight, clear sparkling air and wonderful coloring.

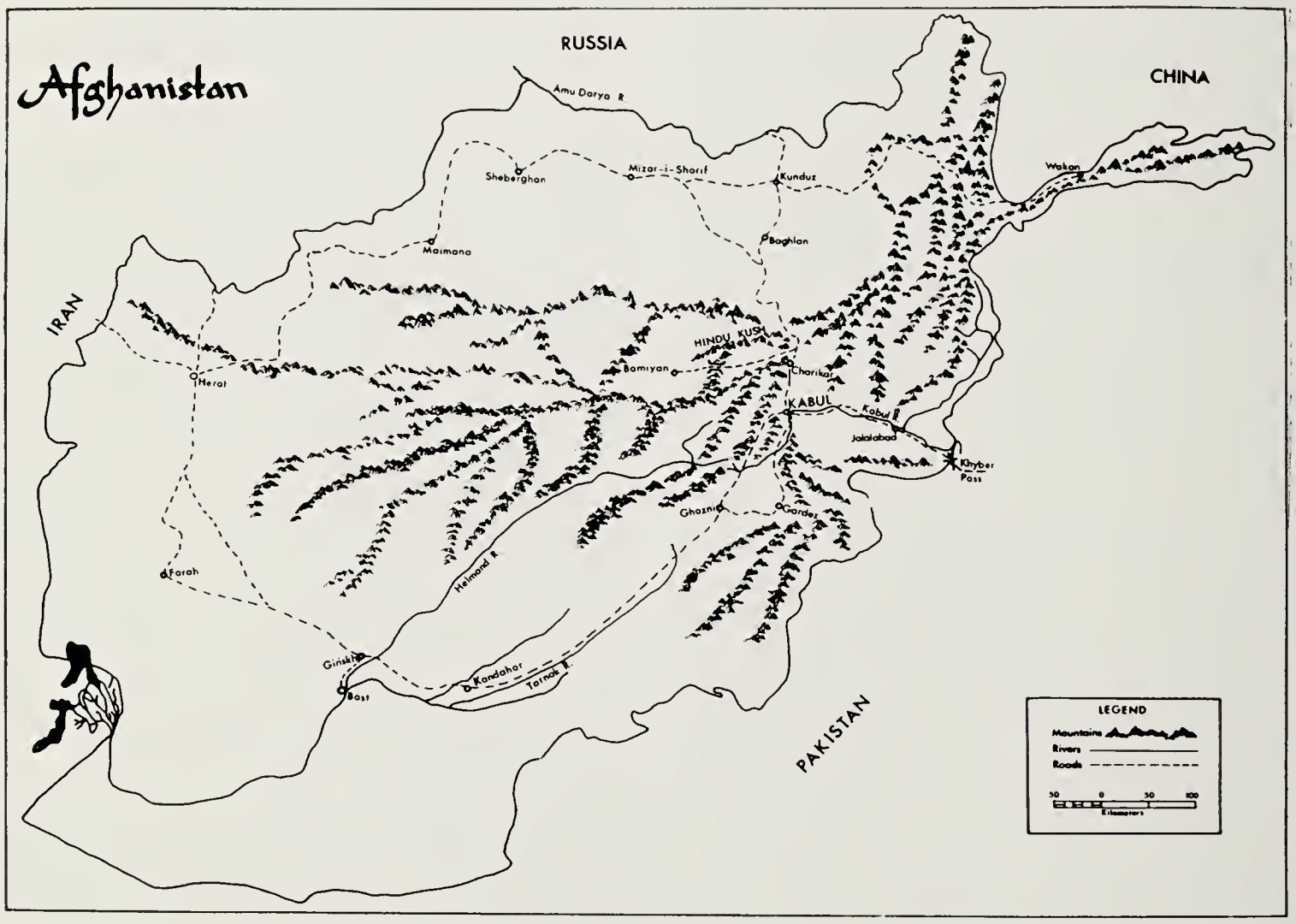

Figure 1. — A map of Afghanistan, showing the location of some of the principal cities and mountain ranges.

The present Kingdom of Afghanistan through which this massive range of mountains runs has an area of some 642,500 square kilometers, about the size of Texas and in the same latitude. Watkins (1963) describes the climate, which is quite different from that of Texas. Without a seacoast and far from any modifying body of water, its temperatures are extreme, influenced not only by seasonal winds but also by the variations in altitude from the lowlands (470 meters) to the highest mountains of the Pamirs and Hindu Kush which rise as high as 7,000 meters. The clear, dry air on a summer day makes the treeless plains swim with sparkingly deceptive mirages leading the traveller hopefully over more simmering miles of rock and sand in search of ever elusive water. No sea breeze or large moderating lake relieves the Helmand Valley, the Seistan Desert, or the low Amu Darya River basin along the north frontier. Forests have gone from most of the countryside, if they ever existed there. The summer sun, unmodified by rain for unbroken miles, beats mercilessly on the land, while cold winds, and, in the higher reaches, snow and ice lash the countryside in winter.

The annual rainfall for the Kabul area, according to 50-year records kept at the British Embassy, is 10-12 inches. Most of the rain comes during the month of April. Westerly winds bring the rain from the Mediterranean 5000 kilometers distant. During the summer there are occasional rain storms of very short duration, following the many dust storms. For all practical purposes, there is no additional moisture for plant life from the end of April until the December snows.

Temperatures in the summer rarely exceed 38° C in the Kabul area and may be as low as —10° C for short periods in the winter. During the fall, temperatures gradually are lowered until the winter range is reached. In the spring there is a gradual warming until maximum temperatures are reached in June.

Kabul, the capital, is located in the east, central part of Afghanistan. (Fig. 1) It lies at an elevation of 1830 meters and is surrounded by steep hills rising another 300 to 600 meters. (Fig. 2) The city completely surrounds an inner range of hills, having three main peaks and running in an east-west direction. The eastern edge of Kabul is bounded by another range called the Sher-i-Darwaza. The two ranges of hills are separated by the narrow gorge of the Kabul River. The Sher-i-Darwaza rises at the Bala-i-sar Fort and continues southward for about eight kilometers, with three main peaks. To the south of this range are a few lower ridges, separating the Kabul valley from the adjoining Logar valley. To the southwest of Kabul and about eleven kilometers from the center of the city is a range of hills rising to a height of 2400-2500 meters. The Kabul valley stretches to the west nearly 32 kilometers from the city and is bounded here by the Paghman range, rising to a height of 5000 meters, and roughly parallel to the Sher-i-Darwaza. Lower hills, 150 to 300 meters above the valley floor, cut off the Kabul valley from the Panshir valley about 11 kilometers to the north.

These hills near Kabul are quite steep and rocky, with few large areas of soil. (Fig. 4) Most of the rock is a mica schist, ranging in color from black, through grey to reddish brown. The schist breaks quite readily into smaller, flat rocks. In the rock-strewn areas the majority of the rocks are 30 centimeters or less in diameter. There are also some igneous rocks such as granite and marble.

Most sites are greatly disturbed. The pressure of both man and beast on the present plants is extremely great. In the early spring the hills are pastured by sheep and goats. As the larger, unpalatable vegetation dies later in the season, it is cut and used for fuel. Rarely, except in mid-winter, does one find these hills free from men or beasts.

Study Methods — Field Data

In the fall of 1963, the author made a preliminary survey of plants on some of the hills surrounding Kabul. It was surprising to see how many living plants remained. There were some shrubs and many herbaceous species with spines. Some of the Compositae were blooming and seeding until the first part of November, a full two weeks after killing frosts had occurred in Kabul. These plants had survived a period of six months without rainfall and with daytime temperatures up to 38° C.

Two years later a transect was established on the Sher-i-Darwaza, reaching a height of 2220 meters. This was called transect 1. (Fig. 2) With the use of an aneroid barometer, thirteen plots were established with elevation differentials of every 30 meters along the transect.

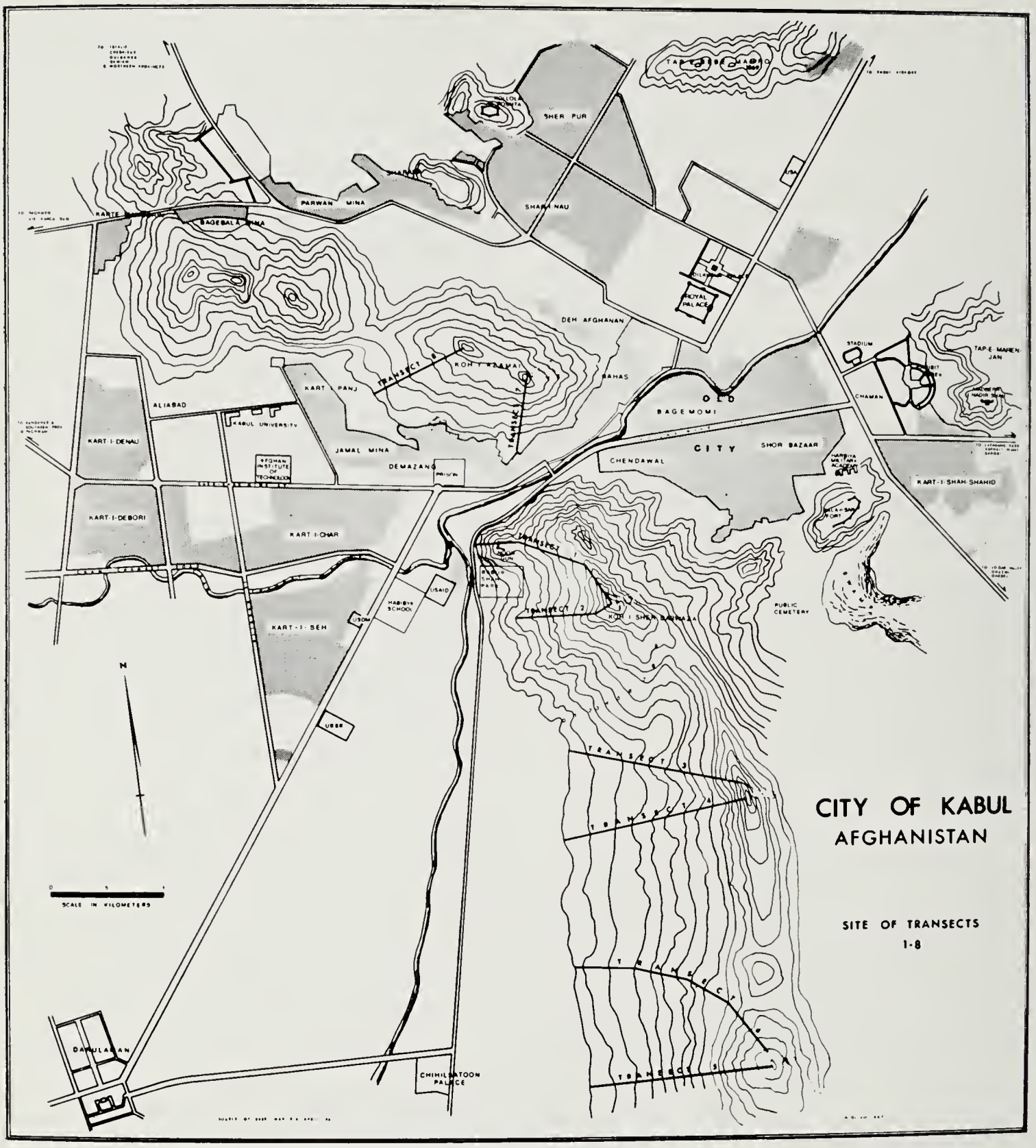

FIGURE 2. — A map of the city of Kabul, Afghanistan, showing the location of eight transects and some of the principal buildings and hills.

An area of 1 by 10 meters was sampled at each location. These sample plots, as far as possible, were located at right angles to the main transect which ran from the base of the hill to the top.

Frequency and cover data were taken. The plants were usually so scattered that no attempt was made to collect density data. A total of 107 different species were collected from this transect. These are listed in Table 5.

In the spring of 1967 nine additional transects were established with a total of 107 study plots. Eight of these transects are shown in Figure 2. The ninth and tenth transects were located on a hill southwest of Kabul about 13 kilometers. These last two transects were selected because this was the only hill in the entire area with a few trees at the top. An additional 22 species of plants were collected from these nine transects, making a total of 129. (Table 5).

The number of study plots per transect varied according to the elevation of

the hill where the transect was located. Transects seven and eight were located on the hills surrounded by the city. The lower reaches were badly disturbed, so data were only taken between 1980 and 2130 meters.

In addition to frequency and cover data, the percentage of solid rock, broken rock and soil was taken at all study plots. The portion designated as soil was arbitrarily chosen for rock and mineral particles less than 5 centimeters in diameter. This "soil" was usually shallow and rocky. The angle of the slope in degrees was also estimated between each of the study plots.

The phenomenon of plants in a vigorous growing state after a six months period without rainfall raised the question of the source of the moisture for growth. The air is dry and there is little dew. Thus the rock itself was thought to be a possible source of water storage. Small, representative samples of the mica schist rock were collected and brought into the laboratory. Most of these have a large variety of lichens on them. The rock pieces were dried in an oven for 3 days at 120° C. Then they were immersed in water for 6 days, with daily weighings. The samples were blotted dry before each weighing. An increase in weight was found, which was probably due to water absorbed by the porous rock.

Study Methods — Data Analysis

The Importance Value (Curtis and McIntosh, 1951) was calculated for some species. From among the total 129 species collected (Table 5), 83 were found in sufficient quantities for frequency data, but only 25 species for cover data (Table 1).

The survey of the area seemed to indicate a homogeneity of vegetation. The Importance Value was a method to show how representative the vegetation was on all the plots. Once the importance of species was established, the similaries of transects were tested by using Motyka's coefficient of community (as used by Bray and Curtis, 1957). This is expressed as:

$$C = \frac{2W}{a + b} \times 100$$

where a is the sum of the quantitative values of all the species in one stand, b is the sum of the quantitative values in another stand being compared, and W is the sum of the quantitative values the two stands have in common for each species. This latter sum is made up from the least values of all the species which are common within the two stands being contrasted. A matrix or table of W values for a single transect as compared with all other transects was prepared.

There have been a number of methods devised for ordering the transects. The purpose of such an ordering is to place the transects in a sequence according to their similarity. Clausen's (1957) method seemed best adapted for the purposes of this study. As shown in Figure 3, transect 1 and transect 8 were the reference points of the gradient.

The percentages of rock, soil, cover and degrees of slope were compared by elevations or plots (Table 2) and correlations were made between these parameters. Table 3 shows these comparisons by transect. The basic outline of the transects is shown in Figure 4.

Results and Discussion

Most of the plants found on the hills were relatively small, and thus the relative cover values were considerably smaller than the relative frequency values. However, since this was true in most of the study plots, the differences are representative of the vegetation of these transects. As Table 1 shows, average cover was only 5.5% for the entire area.

The Importance Values of these species were from 5.64 to a low of 0.87. There was an average difference of 0.19 in the Importance Values of the first 19 species, so these were considered the dominant plants. The difference between *Stachys* and *Thallictrum* was 0.84 in Importance Value, so this was used as the division point between dominants and non-dominants. The remaining six species (Table 1) normally appear in the spring, and they do not persist throughout the entire growing season as do the dominants. The Importance Value of the dominants was 85.88

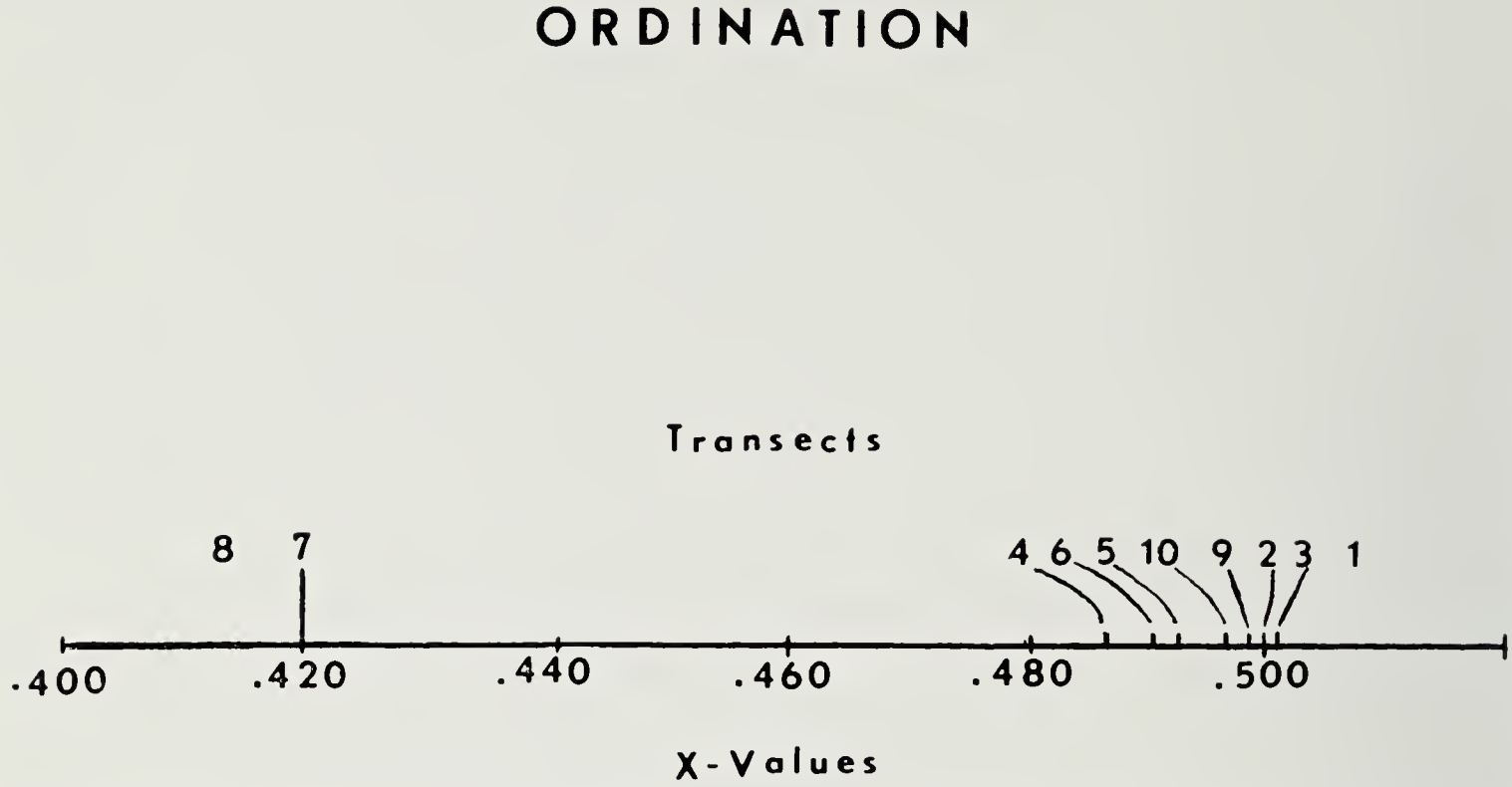

FIGURE 3. — X-values for the ordination of ten transects located on hills surrounding Kabul, Afghanistan.

for all transects, while that of all other vegetation was only 29.59.

The ordination of the transects was completed with the formation of a matrix of X values (Clausen, 1957). The results are shown in Figure 3. Transects 1 and 8 were the reference points, showing the greatest dissimmilarity. Transect 7 was close to Transect 8 in X value. All of the other transects were very close in X values, showing a high degree of similarity in vegetational composition. There was an average X value difference of only 0.002 between transects 3, 2, 9, 10, 5, 6 and 4 and an average X value difference of only 0.067 between transects 4 and 7. A highly homogeneous plant composition in all transects is indicated from these data.

Comparisons were made of percentages of rock, soil, cover and degrees of slope by elevations (plots) and by transect. The means for both kinds of comparison are very close. (Tables 2 and 3). Transect 1 was generally steeper than all other transects, while transects 4 and 10 were slightly steeper than transect 9. The plots at the lower elevations were

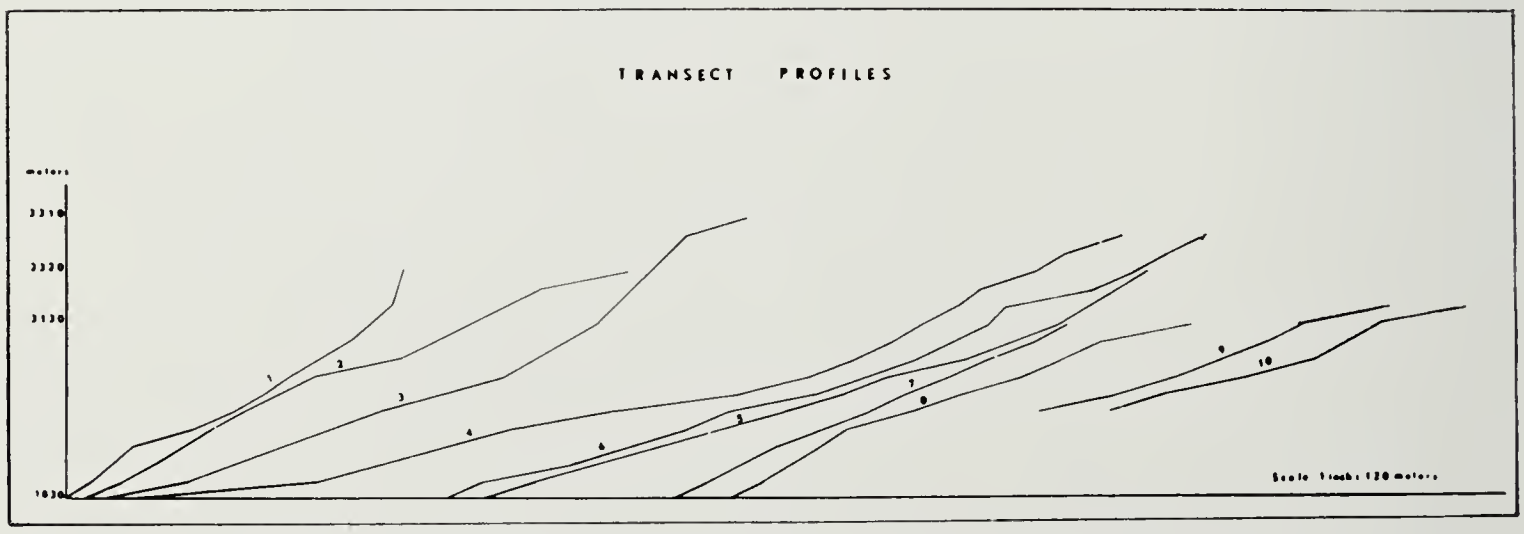

FIGURE 4.—Profiles of ten transects located on hills surrounding Kabul, Afghanistan.

TABLE 1.—IMPORTANCE VALUES OF THE DOMINANT SPECIES FOUND ON TEN TRANSECTS ON HILLS NEAR KABUL.

Species	Transects										
	1	2	3	4	5	6	7	8	9	10	Av.
Cousinia Sp. 1	2.6	5.1	5.0	6.1	4.8	4.8	10.1	10.0	3.7	4.2	5.64
Bromus Sp. 1	3.4	4.8	4.6	5.9	4.6	4.1	9.9	9.7	3.7	4.1	5.44
Bromus Sp. 3	3.2	4.7	4.2	5.8	4.6	4.1	9.9	9.7	3.6	4.1	5.39
Bromus Sp. 2	3.2	4.7	4.1	5.6	4.3	3.9	9.7	9.6	3.5	3.9	5.25
Artemisia Sp. 1	3.4	4.8	2.8	6.6	4.7	4.6	8.3	8.1	3.7	4.2	5.10
Polygonum Sp. 1	3.5	4.4	3.3	4.2	4.0	4.3	9.7	9.7	3.6	4.1	5.08
Cousinia Sp. 3	3.7	2.5	4.9	4.6	4.7	4.8	8.4	8.2	3.7	2.9	4.84
Cousinia Sp. 2	1.3	4.9	4.6	3.6	4.5	3.7	9.7	9.7	3.4	2.8	4.78
Stipa szowitsiana	2.9	3.9	3.7	4.6	4.6	3.6	8.2	8.1	3.7	4.1	4.74
Nepeta bracteata	2.6	4.7	2.4	4.4	2.0	2.3	8.1	8.0	3.5	4.1	4.21
Artemisia Sp. 2	3.2	4.6	2.5	1.5	1.1	....	9.6	9.6	3.4	1.9	3.74
Lactuca Sp. 1	2.6	4.8	4.3	5.3	4.4	4.2	.....	.....	2.9	4.1	3.26
Astragalus paghmenensis	3.2	4.3	4.7	5.3	3.1	3.7	.....	.....	3.4	4.0	3.17
Tulipa systola	2.7	3.8	4.1	4.2	4.3	3.9	.....	.....	3.4	3.3	2.97
Filago spatulata	3.3	4.4	3.2	4.4	4.0	2.3	.....	.....	3.5	4.0	2.91
Carex stenophylla	2.4	2.5	2.8	7.0	1.4	3.8	.....	.....	3.6	2.2	2.57
Eremurus stenophyllus	2.6	2.4	3.2	3.1	3.9	3.9	.....	.....	1.7	2.7	2.35
Euphorbia Sp. 1	3.2	3.8	3.1	3.0	1.1	2.1	.....	.....	2.7	3.3	2.23
Stachys parviflora	1.3	0.8	3.5	3.4	2.7	3.2	.....	.....	3.3	3.9	2.21
Thallictrum sp.	0.7	2.4	3.4	2.6	1.9	2.7	.....	.....	....	....	1.37
Achusa italica	2.1	1.4	1.8	0.7	1.1	1.9	.....	.....	2.7	1.9	1.36
Anemone sp.	1.3	1.8	0.8	2.8	1.9	3.1	.....	.....	0.5	0.6	1.28
Ferula barbeyia	3.3	3.9	2.1	0.8	0.5	2.1	.....	.....	....	....	1.27
Corydalis sp.	1.3	1.8	2.3	0.4	1.9	1.6	.....	.....	0.5	....	0.98
Delphinium cyphoplectrum	1.8	2.1	1.4	0.4	1.1	1.9	.....	.....	....	....	0.87
Sub Totals	64.8	89.3	82.0	96.3	77.0	80.6	101.6	100.4	67.7	70.4	83.01
Other Plants	41.0	17.6	21.9	14.3	27.4	27.0	1.9	3.0	36.5	33.9	22.46
Bare Ground	94.2	93.1	96.1	89.4	95.6	92.4	96.5	96.6	95.8	95.7	94.53
Totals	200	200	200	200	200	200	200	200	200	200	200

TABLE 2.—COMPARISON OF PERCENTAGE OF ROCK, SOIL, COVER, AND DEGREES OF SLOPE BY ELEVATIONS (PLOTS).

	Plot Numbers																Correlations	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Mean	
Rock	47	66	76	77	75	75	78	77	78	87	77	88	84	88	94	85	78	Rock:cover .955
Soil	53	34	24	23	25	25	22	23	22	17	23	12	16	12	6	15	22	Soil:cover .947
Cover	10	5	6	6	5	6	5	5	4	4	4	3	4	4	2	3	5	Cover:slope .587
Angle Slope	17	23	24	19	20	18	20	20	27	29	33	38	33	33	33	15	25	Slope:rock .814

TABLE 3. — COMPARISON OF PERCENTAGES OF ROCK, SOIL, COVER AND DEGREES OF SLOPE BY TRANSECT.

	Transects										Mean
	1	2	3	4	5	6	7	8	9	10	
Rock.....	75	67	88	70	83	83	83	82	73	78	77
Soil.....	25	33	12	30	17	17	17	18	27	22	23
Cover....	6	6	4	8	4	4	4	4	4	4	5
Angle Slope...	40	27	28	19	21	21	24	23	17	19	24

generally less steep than those above 2,100 meters (Plots 9 through 15), where there was a marked increase in steepness. Transect 3, which was the longest and highest, was also the flat-test, having an average angle of slope of just 15 degrees.

There was a highly significant statistical correlation between cover and soil and a highly significant negative statistical correlation between rock area and cover. The correlation between angle of slope and cover was not significant statistically. (Table 2.)

One of the problems of the area as far as plants are concerned is the lack of water during the prolonged periods of drought. In an attempt to find a source of water storage, the lichen-covered rocks were soaked in water after being air dried in an oven. Water was absorbed at the rate of 0.023 grams per hour for the first 44 hours, and then at the rate of 0.001 grams per hour for an additional 97 hours. There was no gain after this

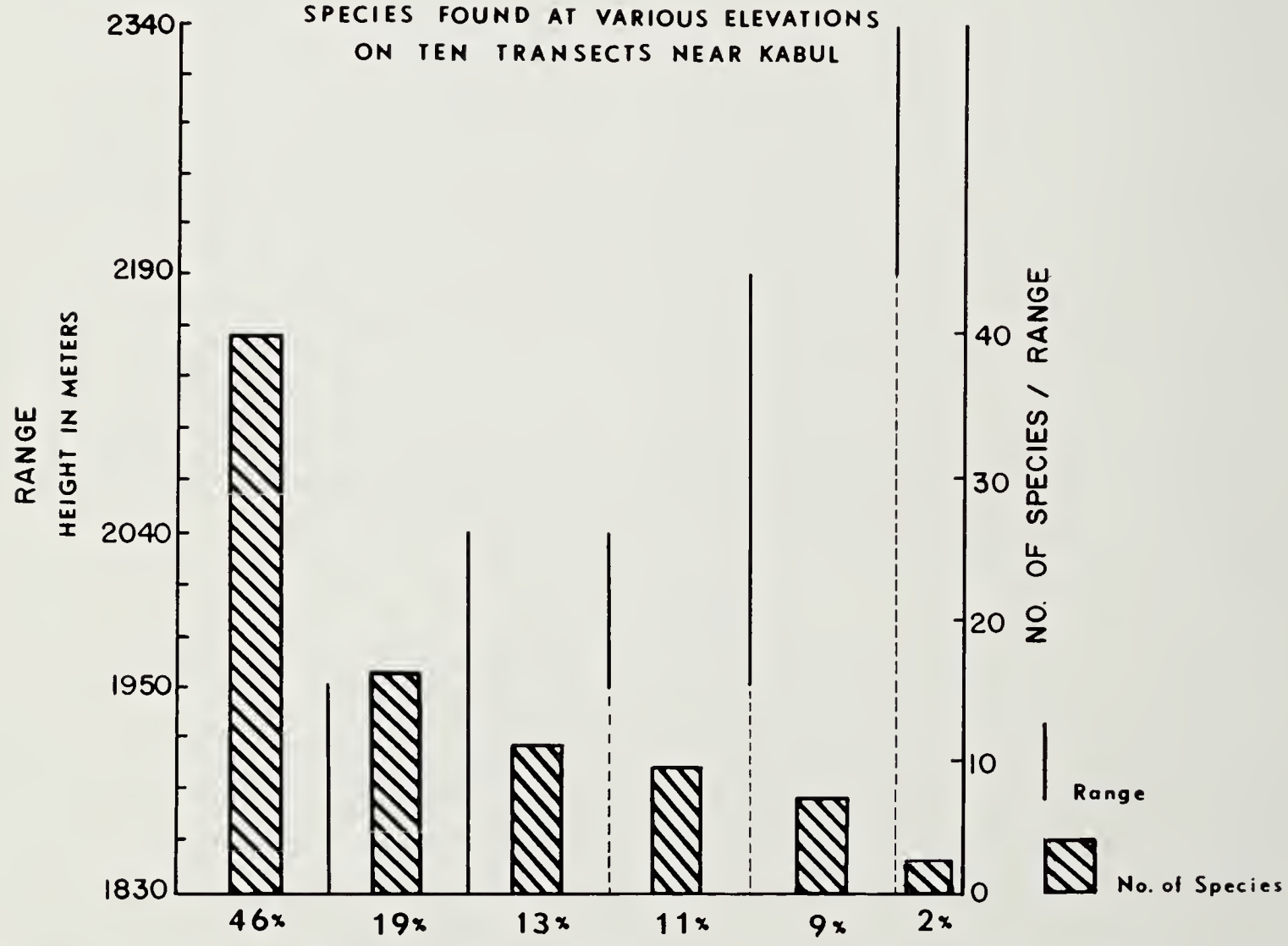

FIGURE 5. — Percentages of species found at various elevational ranges on ten transects near Kabul, Afghanistan.

TABLE 4.—SPECIES FOUND AT VARIOUS ELEVATIONS ON TEN TRANSECTS NEAR KABUL.

I. Species at elevations from 1830 to 2310 meters. (39 species)

†*Cousinia* sp. 1.
†*Bromus* sp. 1.
†*Bromus* sp. 3.
†*Bromus* sp. 2.
†*Artemisia* sp. 1.
†*Polygonum* sp. 1.
†*Cousinia* sp. 3.
†*Cousinia* sp. 2.
†*Stipa szowitsiana*
†*Nepeta bracteata*
†*Artemisia* sp. 2.
†*Lactuca* sp. 1.
†*Astragalus paghmenensis*
†*Tulipa systola*
†*Filago sp.*
†*Carex stenophylla*
†*Eremurus stenophyllus*
†*Euphorbia* sp. 1.
†*Stachys parviflora*
Thalictrum
Achusa
Anemone
Ferula
Corydalis
Delphinium cyphoplectrum
Scrophularia striata
Astragalus sp. 1.
Allium desertorum
Cruciferae sp. 5.
Gagea conjungens
Iris sp. 1.
Senecio sp. 1.
Verbascum
Lithospermum apulum
Goebelia alopecuroides
Matthiola oxyceras
Senecio sp. 2.
Achillea
Iris sp. 2.

II. Species at elevations from 1830 to 1950 meters. (16 species)

Astragalus sp. 3.
Alyssum afghanicum
Poa bulbosa
Erysimum sp. 1.
Erysimum sp. 2.
Descurainia
Fumaria vaillantii
Hordeum
Calepina
Cruciferae sp. 1.
Hypocheris
Ranunculus falcatus
Ranunculus oxyspermus
Alhagi
Veronica bilobia
Haplophyllum filifolium

III. Species at elevations from 1830 to 2040 meters (11 species)

Linaria ascolonia
Arabidopsis
Gallium pauciflorum
Gallium setaceum
Euphorbia arvalis
Lamium sp. 1.
Malva neglecta
Erodium bryoniifolium
Nepeta sp. 1.
Polygala
Poa sinaica

IV. Species at elevations from 1950 to 2040 meters (8 species)

Astragalus sp. 2
Allium mirum
Eremurus anisopterus
Amygdalus spartiodies
Polypodium
Cynanchum arnottianum
Astragalus sp. 4.
Gagea sp. 1.

V. Species at elevations from 1950 to 2190 meters (7 species)

Crassulaceae sp. 2.
Ephedra
Statice gilesii
Gramineae sp. 3
Geranium dissectum
Polygonum sp. 2.
Artemisia sp. 3.

VI. Species at elevations from 2190 to 2310 meters (2 species)

Cousinia sp. 4.
Tulipa sp. 1.

† Dominant plants.

time. The resulting 1.3 percent gain in weight, due to the absorption of water, is equivalent to 44 liters of water absorbed per cubic meter of solid material. This may be a significant factor in the retention of water over long dry periods, in addition to amounts of water held in the soil. However, further investigation is needed, as the rock samples were quite small.

Some of the plant species seemed to be found in elevational zones. (Fig. 5.) The lists of species by elevational range are given in Table 4. From the group of 83 species for which data were collected, 39 species, including the dominants, were found at all elevations (1830 to 2310 meters). There were 16 species which were found only at lower levels, up to 1950 meters. Another group of 11 species ranged from 1830 meters to 2040 meters. A fourth group of 8 species was found between 1950 and 2040 meters, with 7 species found from 1950 to 2190 meters. A sixth group of only 2 species was found between 2190 and 2310 meters.

TABLE 5.—PLANTS COLLECTED ALONG TEN TRANSECTS IN KABUL, AFGHANISTAN DURING 1965 - 1967

BETWEEN 1830 - 2310 METERS

Collection Number	Plant Name
	EPHEDRACEAE
38	*Ephedra intermedia* Shrenk et May
	POLYPODIACEAE
53	*Polypodium* L. sp.
	GRAMINEAE
11	*Bromus* L. Sp.1
76	*Bromus* L. Sp. 2

TABLE 5.—Continued.

Collection Number	Plant Name
	GRAMINEAE—Concluded.
98	*Bromus* L. Sp. 3
12	*Poa bulbosa* L.
62	*Poa sinaica* Stend.
57	*Hordeum glaucum* Stend.
72	*Stipa szowitsiana* Trin.
64	Gramineae Sp. 1
77	Gramineae Sp. 2
131	Gramineae Sp. 3
	CYPERACEAE
40	*Carex stenophylla* Wahlenb.
	LILIACEAE
48	*Eremurus stenophyllus* (Boiss. et B.) Baker
49	*Eremurus anisopterus* Regal.
13	*Tulipa systola* Stapf.
130	*Tulipa* L. sp.
18	*Allium desertorum* Forsek.
41	*Allium mirum* Wendelbo
8	*Gagea conjungens* (Pasch.) Wendelbo
122	*Gagea* Salisb. sp.
6	Liliaceae sp.
	IRIDIACEAE
112	*Iris* L. Sp. 1
129	*Iris* L. Sp. 2
	POLYGONACEAE
33	*Polygonum polyinemoides* Jaub. et Spack.
123	*Polygonum* L. sp.
	CHENOPODIACEAE
59	*Chenopodium botrys* L.
	CARYOPHYLLACEAE
47	*Arenaria griffithii* Boiss.
86	*Gypsophia antari* Post.
104	*Silene villosa* Forssr.
	RANUNCULACEAE
25	*Delphinium cyphoplectrum* Boiss.
102	*Delphinium persicum* Boiss.
45	*Ceratocephalus falcatus* (L.) Pers.
31	*Ranunculus falcatus* Linn.
39	*Ranunculus oxyspermus* M. B.
117	*Anemone* L. sp.
119	*Thalictrum* L. sp.
124	Ranunculaceae sp.

TABLE 5.—Continued.

Collection Number	Plant Name
	PAPAVERACEAE
44	*Papaver macrostomum* Boiss. et Huet.
	FUMARIACEAE
32	*Corydalis* Medic. sp.
23	*Fumaria vaillantii* Lois.
	CRUCIFERAE
19	*Alyssium afghanicum* Reich.
56	*Alyssium* L. sp.
105	*Diplotaxis* Dc. sp.
16	*Arabidopsis* (Dc.) Heynh. sp.
22	*Descurainia* Webb & Benth. sp.
14	*Erysimum* L. Sp. 1
15	*Erysimum* L. Sp. 2
50	*Matthiola flavida* Boiss.
17	*Matthiola oxyceras* Dc.
24	*Calepina* Adans. sp.
84	*Cardaria draba* (L.) Desv.
27	Cruciferae sp. 1
106	Cruciferae sp. 2
107	Cruciferae sp. 3
108	Cruciferae sp. 4
116	Cruciferae sp. 5
	CRASSULACEAE
126	*Sedum sexangulare* L.
114	Crassulaceae sp. 1
115	Crassulaceae sp. 2
	SAXIFRAGACEAE
55	Saxifragaceae sp.
	ROSACEAE
110	*Amygdalus spartiodies* Spach.
	LEGUMINOSAE
3	*Goebelia alopecuroides* (L.) Bge.
4	*Astragalus paghmenensis* Sirg. et Reich.
5	*Astragalus* L. sp. 1
70	*Astragalus* L. sp. 2
92	*Astragalus* L. sp. 3
109	*Astragalus* L. sp. 4
95	*Alhagi* Adans. sp.
97	*Trigonella* L. sp.
	GERANIACEAE
121	*Geranium dissectum* L.
82	*Erodium bryoniifolium* Boiss.
	RUTACEAE
111	*Haplophyllum filifolium* (Spach.) Boiss.

TABLE 5.—Continued.

Collection Number	Plant Name
	POLYGALACEAE
100	*Polygala chinensis* L.
	EUPHORBIACEAE
61	*Euphorbia arvalis* Boiss et Heldr.
65	*Euphorbia allepica* L.
99	*Euphorbia turcomanica* Boiss.
83	*Euphorbia* L. sp.
	MALVACEAE
78	*Malva neglecta* Wallr.
	UMBELLIFERAE
35	*Ferula barbeyi* Post.
68	*Prangas popularia* Lindl.
	PRIMULACEAE
63	*Lysimachia punctata* Walt.
	PLUMBAGINACEAE
120	*Statice gilesii* Hemsl.
	ASCLEPIADACEAE
37	*Cynanchum arnottianum* Wright
	BORAGINACEAE
28	*Lithospermum apulum* L.
29	*Anchusa italica* Ritz.
	LABIATAE
52	*Meehania cordata* (Nutt.) Britt.
9	*Nepeta bracteata* Benth.
93	*Nepeta* L. sp.
46	*Lallemantia royleana* Benth.
66	*Lamium* L. sp. 1
79	*Lamium* L. sp. 2
20	*Stachys parviflora* Benth.
101	*Monarda* L. sp.
	SCROPHULARIACEAE
74	*Verbascum* L. sp.
60	*Linaria micrantha* (Car.) Hoffmgg. et Link
26	*Linaria ascolonia* Boiss. et Kotschy
85	*Scrophularia striata* Boiss.
10	*Veronica bilobia* L.
128	*Tripogon spicatus* (Nees.) Ekman
	RUBIACEAE
43	*Galium pauciflorum* Bunge.
67	*Galium setaceum* Lamk.
51	*Rubia gedrosiaca* Bornm.

TABLE 5.—Concluded.

Collection Number	Plant Name
	DIPSACACEAE
69	*Scabeosa olivieri* Coult.
	VALARIANACEAE
118	*Valeriana* L. sp.
	CAMPANULACEAE
90	*Campanula* L. sp.
	COMPOSITAE
1	*Cousinia* Cass. sp 1
2	*Cousinia* Cass. sp. 2
21	*Cousinia* Cass. sp. 3
125	*Cousinia* Cass. sp. 4
7	*Artemisia* L. sp. 1
113	*Artemisia* L. sp. 2
127	*Artemisia* L. sp. 3
30	*Hypochoeris* L. sp.
42	*Senecio coronopifolius* Desf.
36	*Senecio* L. sp.
94	*Lactuca orientalis* Boiss. et Kotschy
71	*Lactuca* L. sp.
75	*Filago spatulata* Presl.
80	*Reichardia picroides* (L.) Roth.
81	*Koelpinia linearis* Pall.
88	*Achillea santolina* L.
96	*Aaronsohnia* Warbg. et Eig. sp.
34	Compositae sp. 1
58	Compositae sp. 2
87	Compositae sp. 3
91	Compositae sp. 4
89	Compositae sp. 5
73	Compositae sp. 6

CONCLUSIONS

From these data we may conclude:

1. There is more plant life on these rocky hills than is apparent from a distance.

2. The plant life is homogeneous in this area.

3. There is a close correlation between the amount of "soil" and the relative cover of the plants.

4. There is little correlation between steepness of slope and the relative cover of the plants.

5. The lower slopes are less steep than the upper slopes except for the extreme ends of the transects at the summits. Here there is a comparatively flat area.

6. Lichen-covered rocks may be able to store moisture.

7. Forty-six percent of the plants do not seem to be affected by altitude, while the remaining 54 percent seem to be so affected.

8. Most of the high elevation plants, except for 2 species, grow equally well at all elevations sampled.

ACKNOWLEDGMENTS

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SOLVENT EFFECTS ON THE O-H STRETCHING BANDS OF ALCOHOLS

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ABSTRACT. — The wavenumber of the O-H stretching vibration has been determined for nine aliphatic alcohols in the vapor phase and in heptane, carbon tetrachloride, carbon disulfide, and benzene. The extent of interaction between the alcohols and solvents has been related by means of the Kirkwood-Bauer equation. Results show that the much more basic benzene deviates markedly from the other solvents in its effect on the O-H band and, in fact, causes these bands to fall at the same wavenumber for all the alcohols studied. The effects of concentration on the monomer, dimer, and multimer bands of methyl alcohol are discussed.

It is well known that the O-H stretching band in the infrared spectra of alcohols occurs at lower wavenumbers in solvents than in the vapor phase. If there is a simple electrostatic interaction of an oscillating electric dipole with its surroundings of dielectric constant D, then the relative shift in going from the vapor phase to a solvent is proportional to (D — 1)/(2D + 1) (Jones and Badger, 1951 and references therein). Usually this is written as (Josien and Fuson, 1954)

$$\frac{\bar{N}g - \bar{N}s}{\bar{N}g} = \frac{\triangle \bar{N}}{\bar{N}g} = C\,\frac{D-1}{2D+1} \qquad (1)$$

where $\bar{N}s$ and $\bar{N}g$ are the stretching wavenumbers in the vapor phase and in a solvent, respectively. Subsequently, it was suggested that the dielectric constant used should be that obtained at high frequency so that D is approximately the square of the index of refraction, n (Josien and Fuson, 1954). Equation (1) then becomes the usual form of the Kirkwood-Bauer equation

$$\frac{\triangle \bar{N}}{\bar{N}g} = C\,\frac{n^2-1}{2n^2+1} \qquad (2)$$

where C is the proportionality constant. In this study, the O-H stretching wavenumbers have been determined for a series of alcohols in the vapor phase and in several solvents to study solvent effects on the O-H stretching fundamental.

EXPERIMENTAL

The alcohols used in this work were either spectral or reagent grade. The solvents used were spectral grade carbon tetrachloride and carbon disulfide and reagent grade benzene and heptane. These chemicals were used without further purification. Solutions varied from about 0.02 to 0.04 molar depending on the alcohol except when studying concentration effects. The solution spectra were obtained using a cell with a path length of 0.41 mm. Vapor phase spectra were obtained in a gas cell of 10 cm path length using pressures of alcohol vapor varying from about 3 to 75 mm depending on the vapor pressure of the

alcohol at room temperature. All spectra were obtained at room temperature.

Infrared spectra were obtained in the range 3800-2800 cm^{-1} using a Beckman IR 8 grating spectrophotometer. The instrument used recorded linearly in wavenumber, and an auxiliary recorder was used for scale expansion. In order to provide absorption maxima for calibration, the spectrum in a given run was scanned from 3800 to 3200 cm^{-1} with the sample cell in the instrument. At 3200 cm^{-1} the cell was removed and a polystyrene film was placed in the instrument. The spectrum was then scanned to 2800 cm^{-1}, recording the polystyrene peaks on the same continuous spectrum which is linear in wavenumber. The polystyrene peaks at 3101, 3083, 3060, and 3003 cm^{-1} were used for calibration. The O-H stretching bands were usually quite sharp and easily positioned to within about ± 2 cm^{-1}. In benzene and in the vapor phase the bands were broader and could be positioned only to within about ± 4 cm^{-1}.

Results

The positions of the O-H stretching bands of several alcohols are shown in Table 1. Some values for the positions of the O-H stretching bands were found in the literature for a few isolated cases, but no systematic determination has been made. For example, the positions of the O-H stretching bands of methyl, ethyl, and isopropyl alcohols in the vapor phase have been reported (Plyler, 1952; Falk and Whalley, 1961). Since the solvent usually used in hydrogen bonding studies is carbon tetrachloride, most of the O-H band positions have been reported in that solvent, and our results are in good agreement with previously reported values (Motoyama and Jarboe, 1966).

Table 1.—Wavenumbers of O-H Stretching Bands of Alcohols in Several Solvents and in the Vapor Phase.

Alcohol	Solvent[1]				
	Vapor	C_7H_{16}	CCl_4	CS_2	C_6H_6
Methyl	3687	3649	3642	3626	3607
Ethyl	3680	3642	3634[2]	3620	3603
n-Propyl	3677	3639	3636[2]	3621	3605
i-Propyl	3655	3631	3624	3612	3608
n-Butyl	3673	3641	3634	3619	3607
sec-Butyl	3658	3633	3627[3]	3613	3604
t-Butyl	3648	3620	3615	3603	3604
Cyclopentyl	3658	3630	3627	3611	3604
Cyclohexyl	3651	3626	3623	3610	3601

[1] Probable error about ± 2 cm^{-1} in all solvents except benzene. Error in vapor phase and benzene about ± 4 cm^{-1}.
[2] Motoyama and Jarboe (1966).
[3] Kuhn (1952).

The infrared spectra of methyl alcohol at several concentrations are shown in Figure 1 for carbon tetrachloride solutions and in Figure 2 for carbon disulfide solutions.

Discussion

At concentrations less than about 0.05 molar, the infrared spectrum of an alcohol in an inert solvent shows

only one peak, characteristic of the monomer alcohol. If the concentration is increased to 0.10 to 0.15 molar, peaks begin to appear at lower wavenumbers corresponding to alcohol molecules bonded in dimers. At still higher concentrations, peaks appear at still lower wavenumbers which correspond to O-H groups in higher aggregates (Van Ness, *et al.*, 1967). Figures 1 and 2 clearly show these characteristics for methanol in carbon tetrachloride and carbon disulfide.

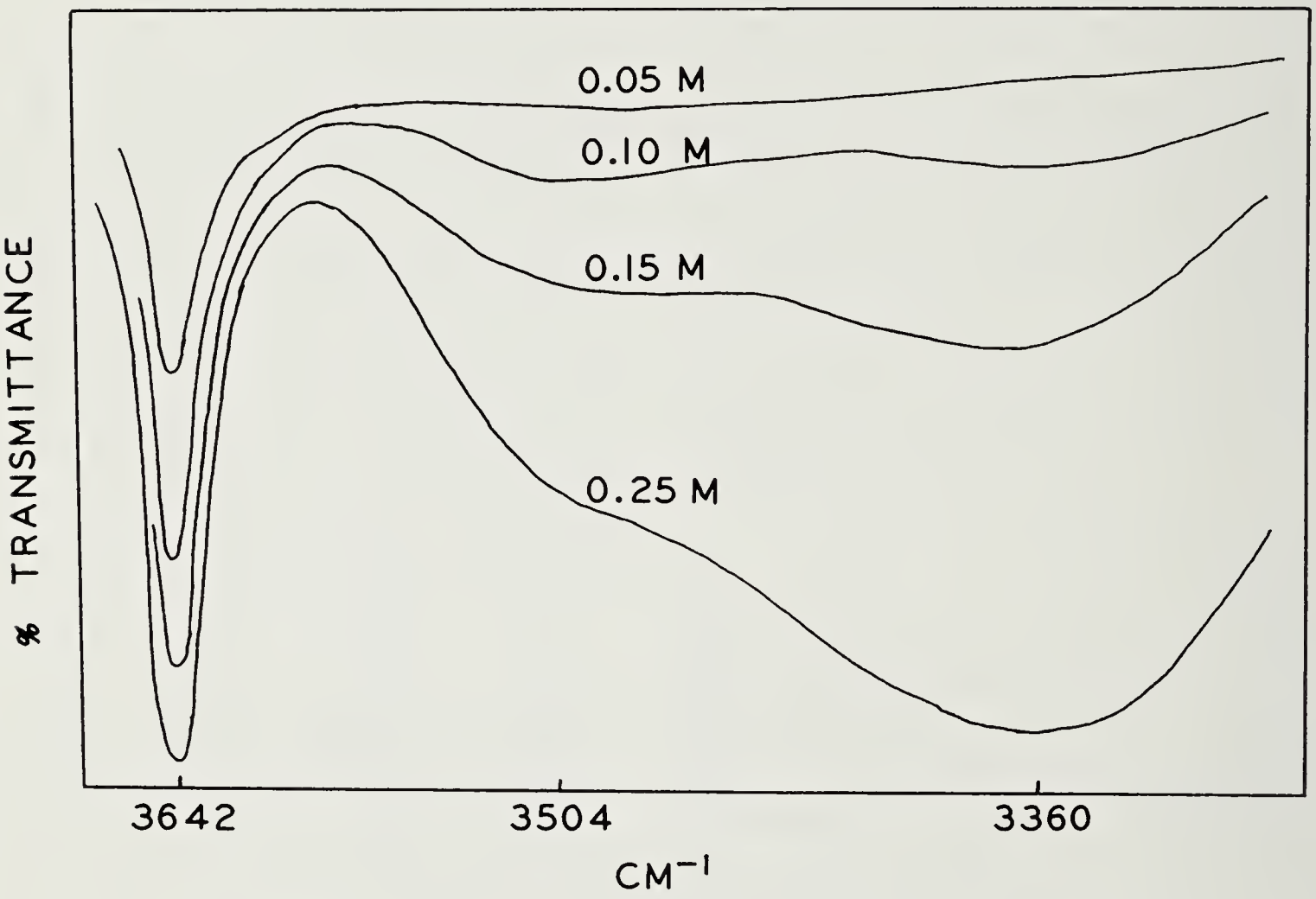

FIGURE 1. — Spectra of solutions of methyl alcohol in carbon tetrachloride.

Qualitatively, there are some interesting comparisons between the behavior of methyl alcohol in CCl_4 and CS_2. As can be seen from a careful study of the spectra in Figures 1 and 2, the curves in CS_2 are not only displaced toward lower wavenumbers but also are not exactly superimposable. In addition to the monomer O-H peak havings its maximum at a different wavenumber, the monomer peak is broader when the solvent is CS_2. This suggests that the O-H group is weakly hydrogen bonded to the solvent since hydrogen bonding leads to much broader O-H bands. It must also be bonded to CCl_4 but more weakly so. No variation in monomer peak position was observed with increasing concentration of alcohol in either solvent. A total of sixteen determinations of the position of the monomer O-H band showed a deviation from the mean of less than ± 2 cm^{-1}.

Another difference in the spectra is in the positions of the peaks corresponding to absorption by dimer and multimer O-H groups. This is more surprising than the difference between

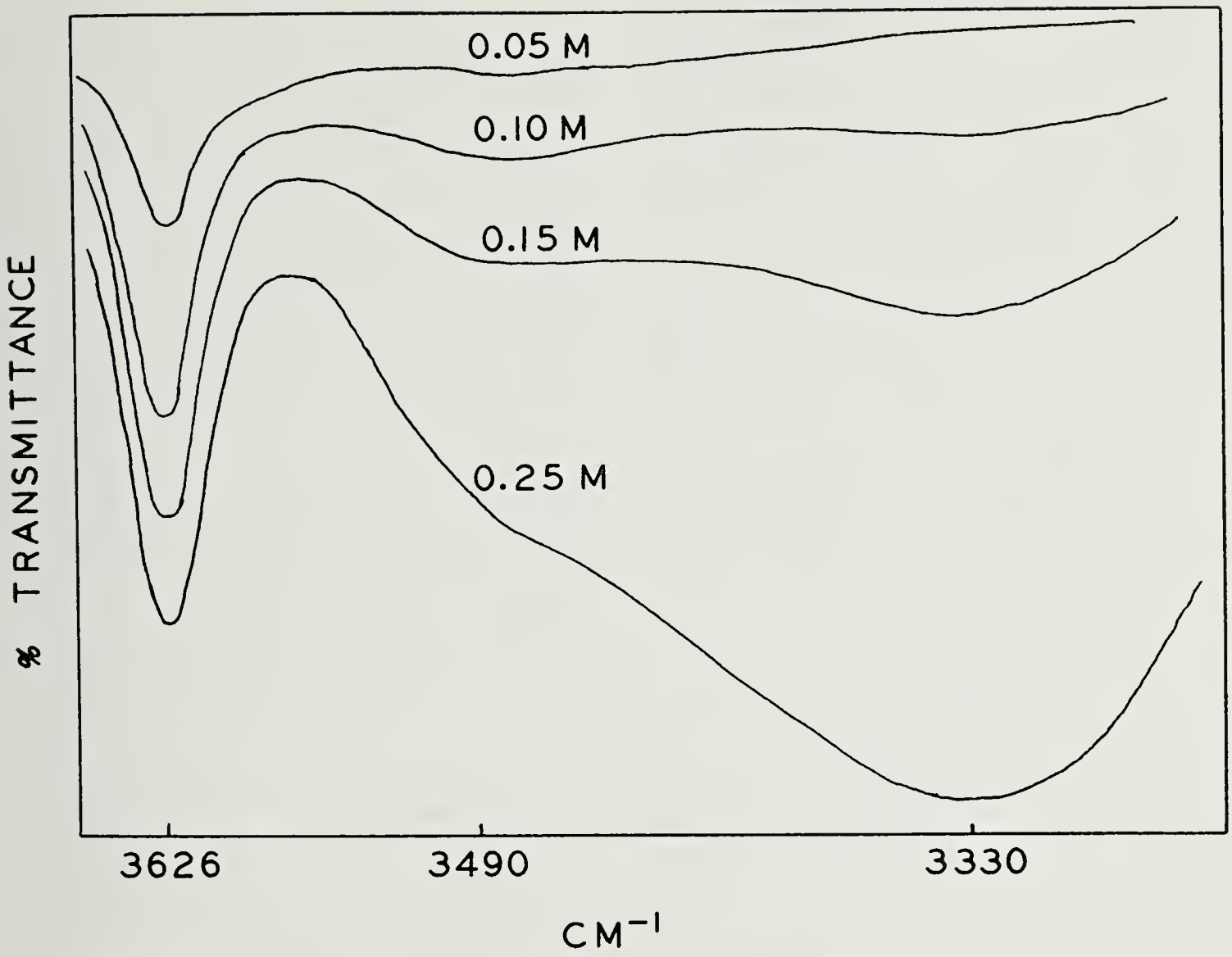

FIGURE 2. — Spectra of solutions of methyl alcohol in carbon disulfide.

the maxima of the O-H monomer peaks. In that case, the difference is due to the differences in electron donor properties of the solvent toward the unattached alcohol molecule. In the multimer unit, the alcohol molecules are linked (Henry, 1959).

```
···O-H···O-H···O-H···O-H···O-H···
 /     /     /     /     /
R     R     R     R     R
```

Shifting the absorption maxima of the internal O-H groups to lower wavenumbers suggests that CS_2 solvates these multimer units more strongly than CCl_4. Otherwise one would expect that the monomer O-H absorption would be shifted to lower wavenumbers but not the multimer O-H band. In fact, the multimer O-H stretching band is shifted in CS_2 by about 30 cm^{-1} from its position in CCl_4.

When an alcohol molecule is hydrogen bonded to an electron donor molecule in solution, there is a large shift in the O-H stretching band to lower wavenumbers. In the gaseous state at low pressures the O-H bond is entirely free of all effects except those of the alkyl group. Between these two extremes there are weak interactions of the O-H group with a solvent, but these are usually not considered as actual hydrogen bonds unless the solvent has considerable ability to donate electrons. In these solvents the O-H stretching band is shifted by 30-80 cm^{-1} from where it might occur in the vapor state and by 10-50 cm^{-1} from where it might occur in an inert solvent.

The data in Table 1 show clearly that although carbon tetrachloride is the usual solvent used in hydrogen bonding studies (Pimentel and McClellan, 1960), it is not completely inert. The O-H band is shifted an average of about 5 cm^{-1} from its position in heptane. It is expected that a saturated hydrocarbon would be more nearly inert than carbon tetrachloride because of the unshared electron pairs on the chlorine atoms and the polarity of the C-Cl bonds. Carbon disulfide is less inert than carbon tetrachloride, shifting the O-H bands an average of about 19 cm^{-1} from their positions in heptane and about 14 cm^{-1} from their positions in CCl_4. These differences in the O-H band positions in the solvents used are presented in Table 2.

TABLE 2.—Differences in Alcohol O-H Band Wavenumber (cm^{-1}) With Solvent: A, Wavenumber in C_7H_{16}; B, Wavenumber in CCl_4; C, Wavenumber in CS_2; D, Wavenumber in C_6H_6.

Alcohol	O-H Band Position Difference, cm^{-1}					
	A-B[1]	A-C[1]	A-D[2]	B-C[1]	B-D[1]	C-D[2]
Methyl	8	23	42	15	34	19
Ethyl	6	22	39	14	31	17
n-Propyl	3	18	34	15	31	16
i-Propyl	7	16	28	12	21	9
n-Butyl	7	22	33	15	26	11
sec-Butyl	6	20	26	14	20	6
t-Butyl	5	17	16	12	11	—1
Cyclopentyl	3	19	26	16	23	7
Cyclohexyl	3	16	25	13	22	9

[1] Probable error about ± 4 cm^{-1}.
[2] Probable error about ± 6 cm^{-1}.

When the solvent is benzene, the O-H stretching band is shifted even farther toward lower wavenumbers, being 33-42 cm^{-1} lower for primary alcohols in benzene than in heptane, 25-28 cm^{-1} lower for the secondary alcohols, and 16 cm^{-1} lower for the one tertiary alcohol used. This indicates that the strength of the interaction of the O-H groups of alcohols with the solvent decreases as primary > secondary > tertiary. Further, it appears that for the primary alcohols used the strength of the interaction varies as methyl > ethyl > n-propyl > n-butyl when the solvents compared are heptane and benzene. This same order is found when the differences in peak positions in CS_2 and benzene are compared. It appears that primary alcohols have their O-H stretching bands shifted by 11-19 cm^{-1}, secondary alcohols by 6-9 cm^{-1}, and tertiary alcohols remain essentially unchanged. Again the shifts indicate that for primary alcohols, the order of decreasing solvent effect is methyl > ethyl > n-propyl > n-butyl. However, the magnitudes of the errors are such that the differences between the primary alcohols fall within the limits of error and this order is not followed for all solvent pairs. Although this trend is followed

for the two solvent pairs, the primary alcohols are all affected to the same extent. Considering the behavior in the other solvent pairs shows that either most of the shifts are about the same or that the errors are large enough to prohibit observing a trend similar to that just described. It does appear that there is a trend showing the solvent effects on the O-H band of alcohols in the order primary > secondary > tertiary and this trend is too large to be experimental error where the difference in solvent is large; e.g., the difference between heptane and benzene or carbon tetrachloride and benzene, shown as columns A-D and B-D in Table 2.

The influence of solvent on the O-H stretching band is further illustrated by a plot of equation (2) for the several solvents (Jones and Badger, 1951). For the four solvents used, the values of the function $(n^2 - 1)/(2n^2 + 1)$ are 0.190, 0.216, 0.228, and 0.263 for heptane, CCl_4, C_6H_6, and CS_2 respectively. Figure 3 shows this plot for the solvents used. The points represented are for methyl alcohol, but the points for all the primary alcohols are almost exactly superimposable. A similar plot for the secondary alcohols gives a line displaced somewhat to the left because of the smaller O-H band shifts for these alcohols. The points for the secondary alcohols are not as nearly superimposable as for the primary alcohols.

It is interesting that in all cases the point for benzene falls far off the line, indicating that the shift of the O-H band is larger than expected for that solvent. Aromatic molecules have considerable basicity (Tamres, 1952)

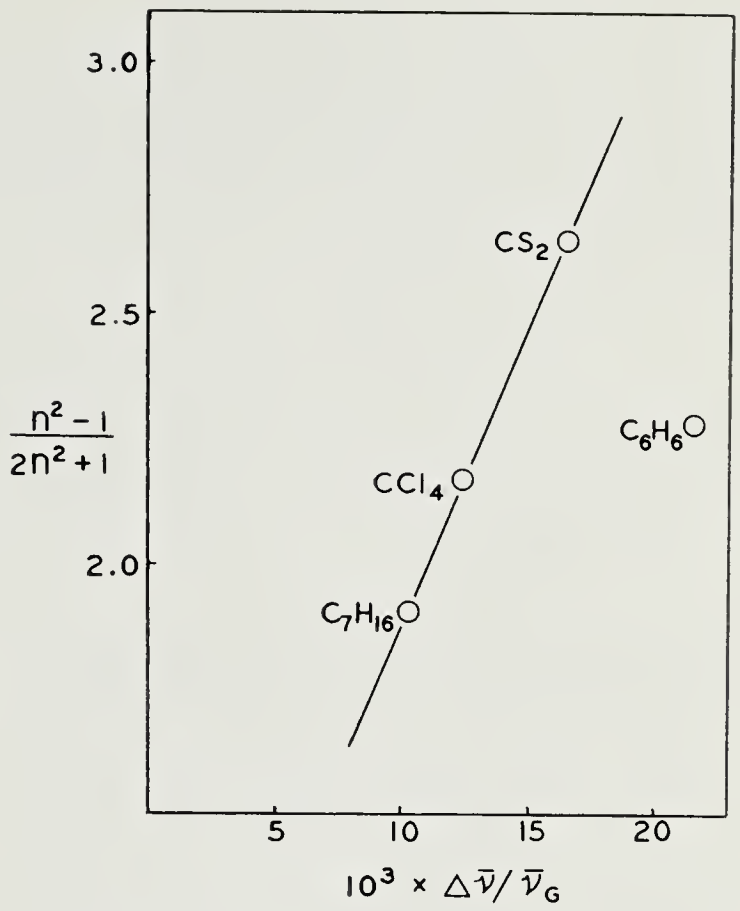

FIGURE 3. — A plot of $(n^2 - 1)/(2n^2 + 1)$ *vs* $\Delta \overline{N}/\overline{N}_g$ for methyl alcohol in several solvents.

and this is reflected by the larger O-H shift.

One further point should be mentioned. In the vapor, heptane, CCl_4, and CS_2, methyl alcohol has the O-H stretching band at 3687, 3649, 3642, and 3626 cm^{-1} respectively, while those for t-butyl alcohol are 3648, 3620, 3615, and 3603 cm^{-1}. All the other O-H bands falls between these two extremes. In benzene solutions, all the O-H bands fall at the same position, 3604 ± 4 cm^{-1}. Thus, it appears that benzene removes the effect of the alkyl group on the O-H stretching band. Assuming that the O-H groups interact equally with benzene molecules, this means that solvation of the alkyl groups must create the same electronic environment of the O-H group in all the alcohols. This behavior is currently under investigation using nuclear resonance techniques.

Acknowledgments

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THE REACTIONS OF 2-NITROSO-1-NAPHTHOL-4-SULFONIC ACID WITH NITRATE IN ACIDIC SOLUTIONS

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ABSTRACT. — The reaction of excess nitrate with 2-nitroso-1-naphthol-4-sulfonic acid in hot, aqueous, perchloric acid solutions forming 2,4-dinitro-1-naphthol has been studied to establish if it is possible to adapt the reaction to the determination of nitrate in natural waters. The reaction was found to occur under optimum conditions of heating at 50° C, in 4.9 *M* $HClO_4$, for 35 minutes. Although 2,4-dinitro-1-naphthol is the product formed when the nitrate ion to 2-nitroso-1-naphthol-4-sulfonic acid molar ratio is 2 or greater, when the ratio is 1 or less, 2-nitro-1-naphthal-4-sulfonic acid is the product. Solutions of 2-nitro-1-naphthol-4-sulfonic acid, prepared under reaction conditions similar to those above, conformed to Beer's law over a nitrate (as nitrogen) concentration range of 0.3 — 13 ppm (under conditions of absorbance measurements), at both 221 and 260 mμ.

Many trace impurities, such as heavy metals and nitrate ions, in water used for drinking purposes, are toxic. More than 45 parts per million (ppm) of nitrate (as N) is often fatal to infants. Over prolonged periods of time, 15 to 20 ppm nitrate (as N) is frequently fatal to livestock. Concentrations of nitrate in natural waters which will not cause death interfere with reproduction, weight gain, and milk production in dairy cattle.

Because of the toxic properties of the nitrate ion, it is advantageous to have a rapid, accurate, and simple method for its determination in water. Existing methods are complicated by several factors including the necessity of prior separations for the removal of interfering substances, use of prolonged evaporation steps either for concentrating to attain minimal concentration levels or to obtain a non-aqueous reaction media, and by many other tedious procedures.

Knowing that 2-nitroso-1-naphthol-4-sulfonic acid reacts rapidly with excess nitrate ion in hot, acidic, aqueous media in near quantitative yields to form 2,4-dinitro-1-naphthol (Witt and Kaufman, 1891), the development of a simple method for the determination of nitrate was thought to be possible utilizing this reaction. Such a study is reported here.

EXPERIMENTAL

MATERIALS

Reagent grade chemicals meeting American Chemical Society specifications were used whenever they were commercially available. Other reagents were the best available. The 2-nitroso-1-naphthol-4-sulfonic acid was prepared by nitrosolation of 1-naphthol-4-sulfonic

acid (Witt and Kaufman, 1891; Caskey, 1962): neutralization equivalent, 325.6 (aqueous potentiometric titration with standard NaOH); theor. 325.30. The water used was deionized water prepared from distilled water by passing it through a monobed, self-indicating, ion-exchange resin, Rohm and Haas Amberlite MB3 (Mallinckrodt 3326), and stored free from carbonate. The standard sodium hydroxide was prepared and stored free from carbonate.

A Perkin-Elmer Model 137B Infracord was used to determine infrared spectra, utilizing potassium bromide pellets. The constant temperature bath, equipped with a Precision Scientific Co. Portatemp regulator, was adjustable from 5° C above ambient to 95° ± 0.25° C. A Corning Model 12 pH meter, equipped with a general-use, glass electrode and a fiber-type, saturated calomel electrode, was used for potentiometric titrations. The electrode system was calibrated using buffers prepared from commercially available concentrates. Beckman Model DB and DK-1A recording spectrophotometers were used for absorbance measurements, along with matched, silica, 1.00-, 2.00-, or 10.00-cm, absorption cells.

ELEMENTAL ANALYSES

Quantitative elemental analyses were made by Alfred Bernhardt Mikroanalytisches Laboratorium, 433 Mulheim (Ruhr), West Germany.

PREPARATION OF 2,4-DINITRO-1-NAPHTHOL

2-Nitroso-1-naphthol-4-sulfonic acid, 9.09 g, was dissolved in deionized water, 76 ml, and concentrated nitric acid, 24 ml, added. The mixture was heated to 70 - 80° C and then cooled in an ice bath. The solid formed was removed by filtration, washed with water and air dried; yield, 6.24 g, 95% theor., mp 134 - 136.5° C, dec. 136.5° C. This crude product was purified by dissolving it in dilute, aqueous sodium hydroxide which was then washed with ether. The aqueous solution was acidified with dilute phosphoric acid, the product extracted into ether, and the latter evaporated to dryness. After recrystallization from three solvents — benzene, chloroform, and absolute ethanol — the solid was sublimed under vacuum at 100° C.

PREPARATION OF 2-NITRO-1-NAPHTHOL-4-SULFONIC ACID

2-Nitroso-1-naphthol-4-sulfonic acid, 10.78 g, was placed in a round-bottom flask and treated with trifluoroacetic acid, 200 ml, and 30% hydrogen peroxide, 4.6 ml, forming a slurry which was stirred for 2½ hours. The solvents were volatilized from the slurry keeping the temperature below 46° C: yield, 10.30 g, 96% theor., mp 159 - 160° C, dec. 155° C. The solid 2-nitro-1-naphthol-4-sulfonic acid was dissolved in water, potassium chloride, 5.45 g, added, and the monopotassium salt recrystallized from water.

PROCEDURES

The nitration reaction system was studied under various conditions by preparing series of solutions holding all variables, but one, constant. The absorbance values of the diluted solutions were obtained to determine the effect of the varied, independent variable upon the progress of the nitration reaction. In general, solutions were prepared (three or more trials were run) by pipeting volumes of perchloric acid into volumetric flasks and similarly adding water (and/or sodium nitrate solution). The flasks were placed in the constant temperature bath for 15 minutes. Measured volumes of 2-nitroso-1-naphthol-4-sulfonic acid solution were added; the flasks were stoppered, shaken, and the observation of time started. Concentrations reported pertain to the resulting solutions. At the end of the measured time intervals, ice-cold water was added, the solutions diluted to volume, and the spectra obtained.

The neutralization equivalent of 2,4-dinitro-1-naphthol was obtained using 70 v/v% ethanol as the sample solvent. The solution was maintained at 50° C by using a stirring-hot plate. Other neutralization equivalents were also obtained potentiometrically, but in aqueous solutions at room temperature.

Results and Discussion

2,4-Dinitro-1-naphthol was isolated as a canary yellow, crystalline solid, which, after sublimation, melted sharply: mp 138 - 139° C, dec. 139° C, lit. mp 138° C (Davis, 1922). Mixed melting points with

commercially available material (Eastman Kodak P5168) occurred at 138 - 139° C. A potentiometric titration of sublimed 2,4-dinitro-1-naphthol with aqueous sodium hydroxide (0.1182 *N* with rel. std. dev. 0.055%, 6 trials), yielded a typical titration curve of a weak acid indicating only one acidic group in the molecule: neut. equiv. 236.5; theor. 234.2. Anal. calcd. for $C_{10}H_6N_2O_5$: C, 51.29; H, 2.58; N, 11.96; O, 34.16. Found: C, 51.36; H, 2.70; N, 12.11; O (difference), 33.83.

The characteristics of the ultraviolet spectra of 2-nitroso-1-naphthol-4-sulfonic acid and 2,4-dinitro-1-naphthol are presented in Table 1. The progress of a reaction, where 2,4-dinitro-1-naphthol is formed from 2-nitroso-1-naphthol-4-sulfonic acid, can be followed by a decrease in absorbance at 249 and 261 mμ, and by an increase in absorbance at 299 mμ.

In order to determine the conditions under which this system could be adapted to the determination of nitrate, it was necessary to study the effects of acid concentration, temperature, and time on the chemical system. The conditions for the preparation of 2-nitroso-1-naphthol-4-sulfonic acid indicated the compound was stable in hot, acidic, aqueous solutions for a considerable period of time. However, it was soon observed that the acid concentrations necessary for a reaction between low concentrations of nitrate and 2-nitroso-1-naphthol-4-sulfonic acid caused partial decomposition of the latter. The exact conditions of stability were made the focus of attention.

The effect of temperature on the stability of 2-nitroso-1-naphthol-4-sulfonic acid (2.50×10^{-4} *M*) in 2.90 *M* perchloric acid and heated for 35 minutes, is presented in Figure 1.

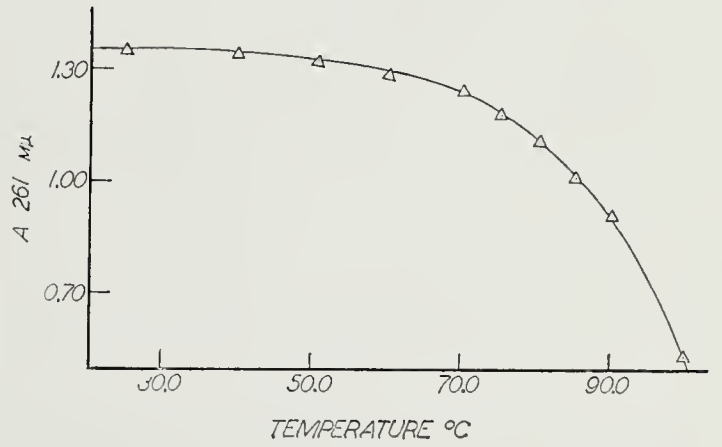

FIGURE 1. — The stability of 2-nitroso-1-naphthol-4-sulfonic acid (2.50×10^{-4} *M*) as a function of temperature when heated in 2.90 *M* perchloric acid for 35 minutes.

TABLE 1. — Ultraviolet absorption spectra

2-Nitroso-1-naphthol 4-sulfonic Acid				2,4-Dinitro-1-naphthol		2-Nitro-1-naphthol 4-sulfonic Acid	
Exp.		Lit.[1]		Exp.		Exp.	
λmax (mμ)	ε	λmax (mμ)	ε[2]	λmax (mμ)	ε[3]	λmax (mμ)	ε[3]
				210	22,400	221	38,700
261	27,000	261	26,385	249	16,800	270	21,300
347	5,000	347	4,451	299	12,300	295	9,610
				375	8,450	387	5,280

[1] (Caskey, 1962).
[2] From slopes of Beer's law plots.
[3] From slopes of Beer's law plots, calculated via IBM 1620 computer from the original data.

The absorbance decrease at 261 mμ became significant at 60° C, indicating significant decomposition of 2-nitroso-1-naphthol-4-sulfonic acid occurred. In 2.90 *M* perchloric acid the temperature must be no higher than 50° C, to insure stability of 2-nitroso-1-naphthol-4-sulfonic acid.

The stability of 2-nitroso-1-naphthol-4-sulfonic acid ([1.72 to 2.50] $\times$ 10^{-4} *M*) as a function of perchloric acid concentration at 50.5° C, and heated for 35 minutes, is presented in Figure 2. The slight negative slope indicated that the increasing concentration of perchloric acid caused only a slight increase in decomposition of 2-nitroso-1-naphthol-4-sulfonic acid. A concentration of 5.0 - 6.0 *M* perchloric acid caused less than a 3% decrease in absorbance. In a similar study conducted at 90° C, in which the solutions were heated for 1 hr, 1.537 $\times$ 10^{-3} *M* 2-nitroso-1-naphthol-4-sulfonic acid was shown to decompose significantly in 0.5 *M* or more concentrated perchloric acid solutions; very little decomposition was observed when the acid concentration was less than 0.1 *M*.

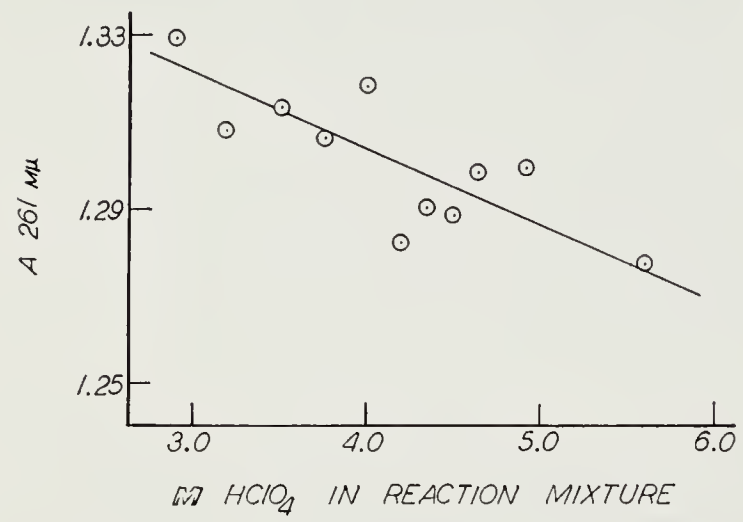

FIGURE 2. — The stability of 2-nitroso-1-naphthol-4-sulfonic acid ([1.72 to 2.50] $\times$ 10^{-4} *M*) as a function of perchloric acid concentration at 50.5° C when heated for 35 minutes.

The stability of 2-nitroso-1-naphthol-4-sulfonic acid (1.92 $\times$ 10^{-4} *M*) as a function of time of heating in 4.91 *M* perchloric acid at 50.5° C is presented in Figure 3. Little or no decomposition occurred during the first 30 minutes, but during the first hour it was about 2% and during the first two hours it was 5%. Longer heating periods led to further decomposition. In a similar study conducted with 1.537 $\times$ 10^{-3} *M* 2-nitroso-1-naphthol-4-sulfonic acid in 2.49 *M* perchloric acid at 94° C, significant decomposition occurred rapidly and amounted to more than 50% after a five minute period of heating.

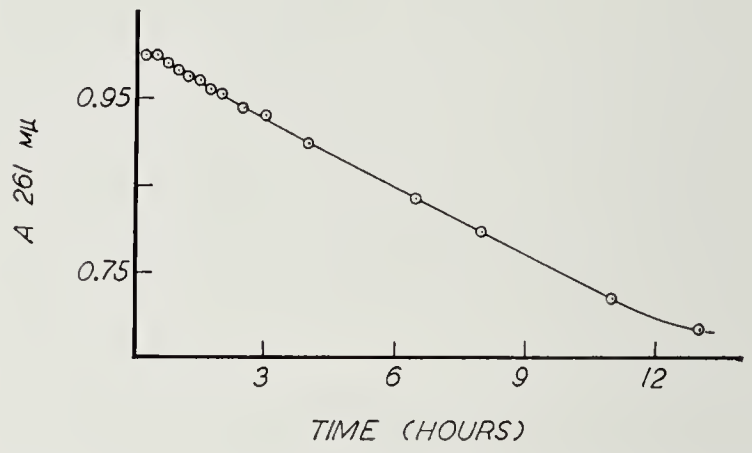

FIGURE 3. — The stability of 2-nitroso-1-naphthol-4-sulfonic acid (1.92 $\times$ 10^{-4} *M*) as a function of time of heating at 50.5° C, in 4.91 *M* perchloric acid.

The nitration reaction was studied using concentrations of sodium nitrate in a one-hundred fold, or greater, mole excess in respect to the concentration of 2-nitroso-1-naphthol-4-sulfonic acid. The conditions of the other variables were similar to those previously employed in the stability studies. No significant formation of 2,4-dinitro-1-naphthol was observed in 2.90 *M* perchloric acid when 2.50 $\times$ 10^{-4} *M* 2-nitroso-1-naphthol-4-sulfonic acid and 2.50 $\times$ 10^{-2} *M* $NaNO_3$ were heated for 35 minutes at temperatures as high as 90° C; the

data, when plotted, yielded a curve almost identical to that of Figure 1. Hence a higher acid concentration or a higher temperature was needed.

The effect of the perchloric acid concentration on the formation of 2,4-dinitro-1-naphthol at 50.5° C is presented in Figure 4, and was studied using solutions containing (1.72 to 2.50) $\times$ 10^{-4} *M* 2-nitroso-1-naphthol-4-sulfonic acid with exactly a one-hundred fold greater concentration of sodium nitrate, respectively, and heated for 35 minutes. It can be seen that although nitration sometimes occurred in 4.0 - 4.5 *M* perchloric acid, concentrations greater than 4.5 *M* were required to insure nitration. The slight decrease in slope of the solid lines was perhaps due to a slight decomposition of 2-nitroso-1-naphthol-4-sulfonic acid.

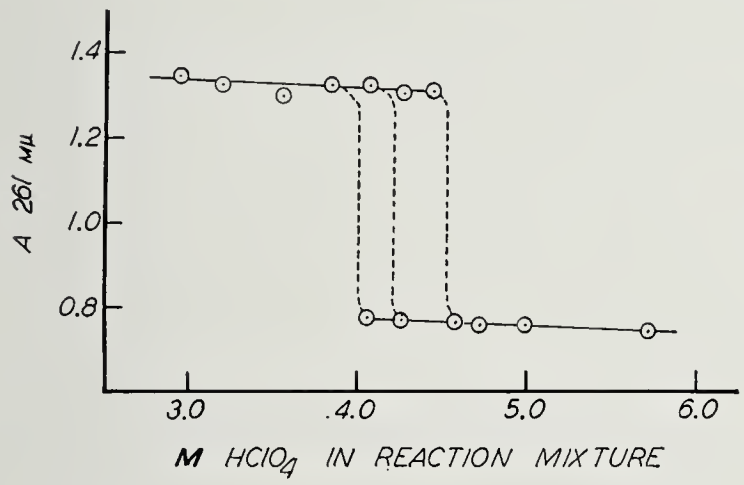

FIGURE 4. — The stability and formation of 2,4-dinitro-1-naphthol, as a function of perchloric acid concentration. The solutions contained (1.72 to 2.50) $\times$ 10^{-4} *M* 2-nitroso-1-naphthol-4-sulfonic acid and exactly a 100 fold greater concentration of sodium nitrate, respectively, and were heated for 35 minutes at 50.5° C.

The effect of time on the formation and stability of 2,4-dinitro-1-naphthol in 4.91 *M* perchloric acid at 50.5° C is presented in Figure 5. Inasmuch as the absorbance at 261 mμ reached a minimum value (the concentration of 2-nitroso-1-naphthol-4-sulfonic acid had reached a minimum value) and the absorbance at 299 mμ had reached a maximum value (the concentration of 2,4-dinitro-1-naphthol had reached a maximum value), the nitration was complete within 35 minutes. It was evident that once the maximum concentration of 2,4-dinitro-1-naphthol was formed, it was essentially independent of time. In a similar experiment using 1.537 $\times$ 10^{-4} *M* 2-nitroso-1-naphthol-4-sulfonic acid and 2.50 $\times$ 10^{-2} *M* sodium nitrate in 2.49 *M* perchloric acid at 94° C, the absorbance at 249 mμ reached a minimum, constant value after 25 minutes, indicating the nitration was complete within 25 minutes, and the product, 2,4-dinitro-1-naphthol was stable in the hot perchloric acid.

The system was studied as a function of nitrate concentration. The pertinent data is presented in Figure 6. When a reaction was observed not to occur in 4.91 *M* perchloric acid, a higher concentration of perchloric acid, 8.44 *M*, at 50.5° C was found, by trial and error, to cause a reaction to occur between 2-nitroso-1-naphthol-4-sulfonic acid and sodium nitrate in a series of solutions containing 7.14 $\times$ 10^{-4} *M* 2-nitroso-1-naphthol-4-sulfonic acid and varying concentrations of nitrate (less than 7.14 $\times$ 10^{-4} *M* sodium nitrate) during the heating period of 45 minutes. A higher concentration of perchloric acid was examined since it had been shown (see Figure 2) to have a smaller effect on the stability of 2-nitroso-1-naphthol-4-sulfonic acid than did longer heating periods or higher temperatures. The absorbance values at 261 and 221 mμ for the

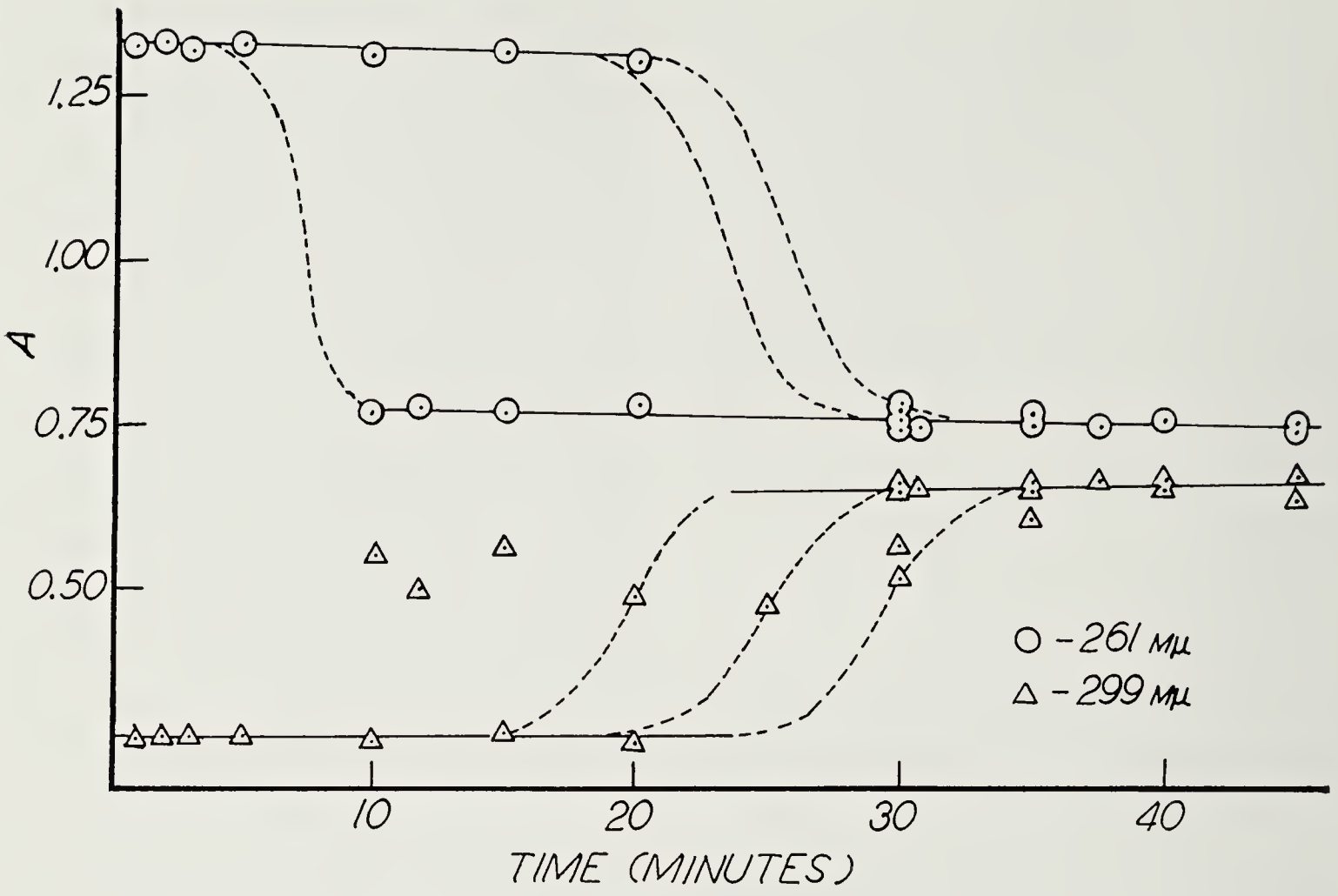

FIGURE 5. — The stability and formation of 2,4-dinitro-1-naphthol as a function of time at 50° C in 4.91 *M* perchloric acid. The solutions contained 1.92 x 10^{-4} *M* 2-nitroso-1-naphthol-4-sulfonic acid and 1.92 x 10^{-2} *M* sodium nitrate.

diluted solutions (plotted as a function of the nitrate concentration in them) gave a linear relationship between 0.3 and 13 ppm nitrate (as N). This study was reproduced in a second similar study.

The spectra of the solutions used to obtain the information presented in Figure 6 indicated that the product of the nitration reaction was not the expected product, 2,4-dinitro-1-naphthol. The new product did not form a complex with cobalt, indicating that the 2-nitroso-1-naphthol functional groups had been affected in some manner. It was noted that both the new nitration product and 2,4-dinitro-1-naphthol had an absorption maximum near 300 mμ. It was also noted that 2-nitro-1-naphthol had been reported by Edwards and Tate (1951) to have an absorption at 270 mμ with a molar absorptivity of 16,700 in ethanol. The new product had an absorption at 270 mμ with a molar absorptivity of 21,300. It seemed reasonable to expect that the new product was an isolable intermediate for the formation of 2,4-dinitro-1-naphthol containing only one

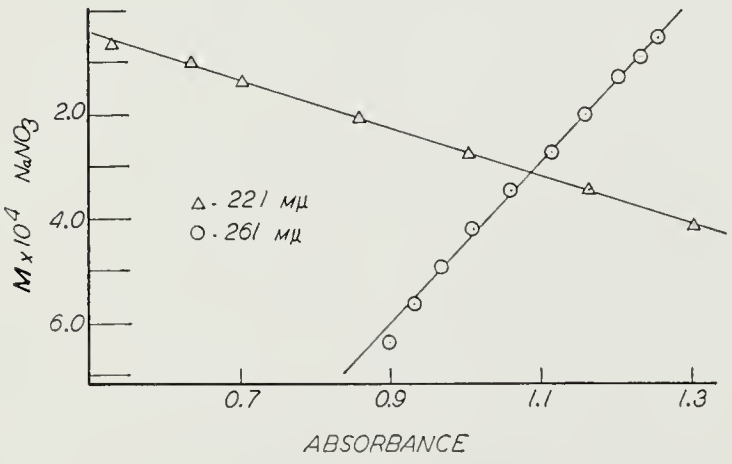

FIGURE 6. — The absorbance of 2-nitro-1-naphthol-4-sulfonic acid as a function of nitrate concentration in 8.44 *M* perchloric acid after heating at 50.5° C for 45 minutes.

nitro group, and the product might be 2-nitro-1-naphthol-4-sulfonic acid.

Using a procedure similar to that described by Emmons and Ferris (1953), 2-nitro-1-naphthol-4-sulfonic acid was successfully prepared from 2-nitroso-1-naphthol-4-sulfonic acid using hydrogen peroxide dissolved in trifluoroacetic acid. Although characterization of 2-nitro-1-naphthol-4-sulfonic acid has not been reported, Witt and Schneider (1901) have reported the partial characterization of several monovalent and bivalent metal salts of 2-nitro-1-naphthol-4-sulfonic acid. The crude product was successfully recrystallized from water as the monopotassium salt. The characteristics of the ultraviolet spectrum are presented in Table 1 and the infrared spectrum in Figure 7. The recrystallized potassium 2-nitro-1-naphthol-4-sulfonate was titrated with aqueous sodium hydroxide (0.1176 N, with rel. std. dev. 0.063%, 5 trials) yielding a typical titration curve of a weak acid indicating only one weak acidic group in the molecule: neut. equiv. 328.8; theor., 325.3; pKa, 4.8; Anal. calcd. for $C_{10}H_6NO_6SK$: C, 39.08; H, 1.97; N, 4.56; S, 10.43; K, 12.72; O, 31.24. Found: C, 39.43; H, 2.10; N, 4.54, 4.71; S, 10.21; K, 12.52; O, (difference), 31.11. Anal. calcd. for $C_{10}H_6NO_6SK{\cdot}H_2O$:$H_2O$, 5.53. Found: H_2O, 5.96, 6.02.

The ultraviolet spectrum of potassium 2-nitro-1-naphthol-4-sulfonate was identical with the spectra obtained from nitration systems where the molar ratio of sodium nitrate to 2-nitroso-1-naphthol-4-sulfonic acid was 1:1. This evidence indicated that the product formed in hot aqueous perchloric acid by the reaction of sodium nitrate with an equivalent, or greater, concentration of 2-nitroso-1-naphthol-4-sulfonic acid was 2-nitro-1-naphthol-4-sulfonic acid. The evidence also indicated that the latter compound was an intermediate in the formation of 2,4-dinitro-1-naphthol from 2-nitroso-1-naphthol-4-sulfonic acid. The apparent random location of some data points in Figures 4 and 5 can also be accounted for in terms of the intermediate formation of 2-nitro-1-naphthol-4-sulfonic acid.

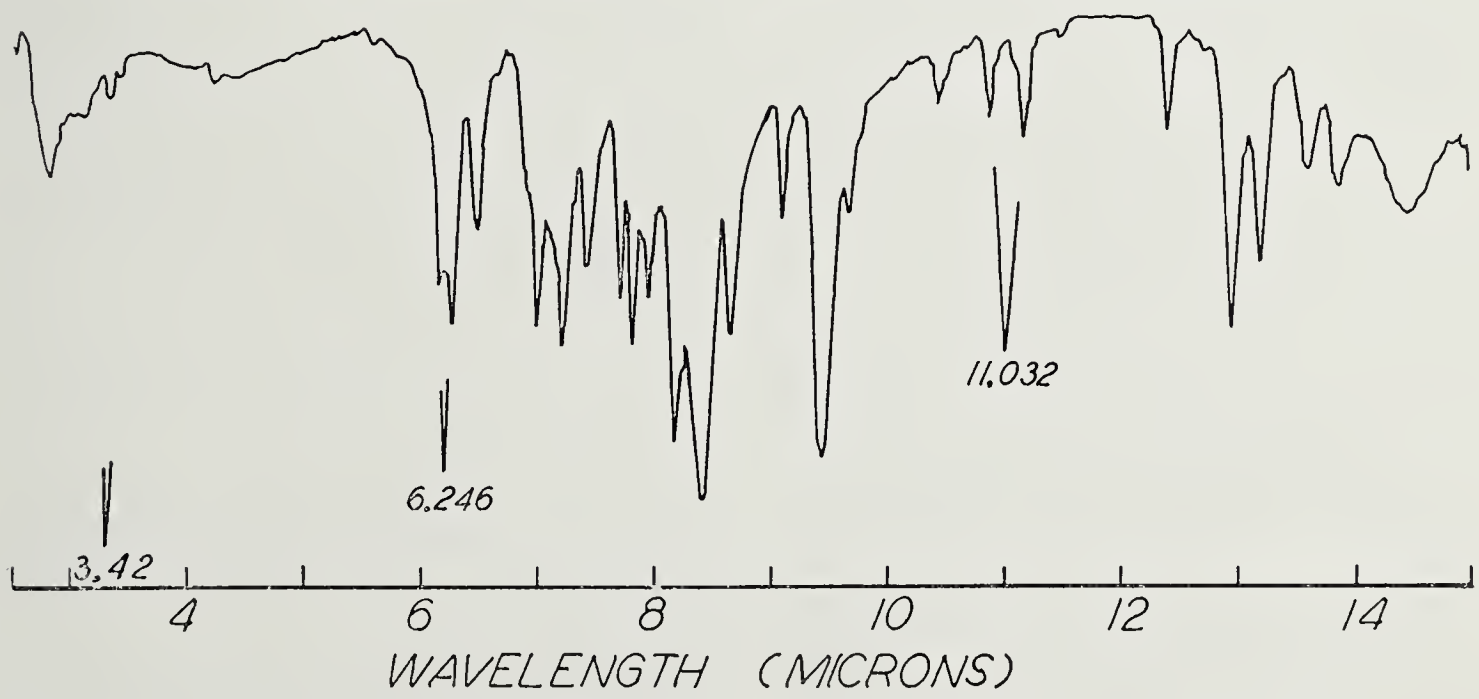

FIGURE 7. — Infrared spectrum of potassium 2-nitro-1-naphthol-4-sulfonate (potassium bromide pellet).

Summary

The stability of the analytical chemical reagent, 2-nitroso-1-naphthol-4-sulfonic acid, has been studied as a function of temperature, length of time of heating, and perchloric acid concentration. It does not significantly decompose when heated for less than 2 hours, in concentrations of perchloric acid less than 4.9 *M*, and temperatures less than 50° C.

The product formed by the reaction of 2-nitroso-1-naphthol-4-sulfonic acid with excess nitrate in hot, acidic, aqueous solutions was isolated, characterized, and clearly reaffirmed to be 2,4-dinitro-1-naphthol.

2-Nitro-1-naphthol-4-sulfonic acid has been prepared by the "nitration" and oxidation of 2-nitroso-1-naphthol-4-sulfonic acid. The potassium salt of 2-nitro-1-naphthol-4-sulfonic acid has been isolated, characterized, and clearly reaffirmed to exist as a monohydrate.

Excess 2-nitroso-1-naphthol-4-sulfonic acid reacts with nitrate ion in 8.44 *M* perchloric acid at 50.5° C within 45 minutes to form 2-nitro-1-naphthol-4-sulfonic acid. The absorbance values at the most intense absorption maxima of 2-nitroso-1-naphthol-4-sulfonic acid and of 2-nitro-1-naphthol-4-sulfonic acid have been shown to be linearly dependent upon the amount of nitrate present. Beer's law is obeyed over a range of 0.3 - 13 ppm nitrate (as N) under the conditions of absorbance measurement.

Preliminary information has been presented which will serve as the basis for the development of a method for the determination of nitrate. The development of a new method for nitrate determinations can be accomplished by delimiting the ideal conditions for the formation of 2-nitro-1-naphthol-4-sulfonic acid. Kirkbright (1966) has outlined and discussed extensively the additional factors which need consideration in the development of new spectrophotometric methods. These factors are under examination in this laboratory and attempts are being made to extend the concentration limits for the determination of nitrate in water.

Acknowledgments

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INFRARED METHOD FOR THE DETERMINATION OF NITROGLYCERIN IN DOUBLE BASE PROPELLANT

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ABSTRACT. — An infrared method is presented for the determination of nitroglycerin in double base propellant. The method utilizes the 12 micron absorption band of nitroglycerin in order to avoid interference from other propellant ingredients which absorb appreciably at 6.02 and 7.85 microns, the normally used wavelengths for obtaining quantitative data for nitroglycerin. Results by this method are compared to those obtained by the titanous chloride method.

Double base solid propellant consists of the two "base" ingredients nitrocellulose and nitroglycerin in addition to various stabilizers, plasticizers, and ballistic modifiers, depending upon the formulation.

The analysis of this type of propellant usually involves the extraction of the propellant sample by an organic solvent such as methylene chloride ($MeCl_2$). This serves primarily to separate nitroglycerin and other organic components from the nitrocellulose which is not extracted. The quantity of material extracted from the propellant by the $MeCl_2$ extraction serves as a routine check on the propellant and is determined by evaporating the extracted $MeCl_2$ solution to a constant-weight residue. This constant-weight extract can be further analyzed for the individual components contained in it.

The nitroglycerin in the constant-weight extract is often determined by the titanous chloride method (Becker, 1933 and Hirschhorn, 1947). This involves reacting the nitroglycerin with ferrous iron, and titration of the ferric iron produced with a standard titanous chloride ($TiCl_3$) solution. This titrimetric method can be quite time consuming, requires close control of temperature, and must be carried out in an inert atmosphere. The $TiCl_3$ titrant solution must be kept in the dark and under an inert atmosphere. It must be restandardized at each usage or at least every other day.

The infrared study reported here was undertaken in order to develop a more simple and less time consuming method of estimating the nitroglycerin content of double base propellant. It was desirable to work with the constant-weight extract rather than attempting to further separate the nitroglycerin inasmuch as this would be even more time consuming. The use of this extract, however, required that consideration be given to the infrared absorption of the other components present in the extract. Infrared spectra were obtained and studied

for each component of the extract. The infrared spectra of several solvents were also considered in the selection of a suitable solvent system for this method.

Nitroglycerin has three major absorption bands; namely, 6.02, 7.85, and 12.0 microns. Infrared procedures have been developed which employ both the 6.02 and the 7.85 micron absorption bands of nitroglycerin (Castelli, 1954; Schwartzman, 1956; Carol, 1958; and Tippett, 1963). These absorption bands are well suited for quantitative measurement since they are sharp and intense. Neither of these wavelengths could be employed for the system being investigated since there were components of the propellant present in the $MeCl_2$ extract which showed appreciable absorption at both 6.02 and 7.85 microns.

Although the 12 micron absorption band of nitroglycerin is of medium intensity and somewhat broad, it was felt that this band could possibly be used for quantitative measurement. The constant-weight extract could have been redissolved in $MeCl_2$, but this solvent shows very intense absorption in the 12 micron region. Benzene was chosen as the solvent because it shows negligible infrared absorption in the 12 micron region and readily dissolved all the components of the constant-weight extract.

Materials and Methods

Preparation of Standards. For reasons of safety, nitroglycerin is often handled as a 50 per cent solution in ethyl acetate. The nitroglycerin used in this study was available in this form and was treated initially by directing a stream of nitrogen into an open beaker containing the nitroglycerin solution. This removed the major part of the ethyl acetate. Removal of the last of the ethyl acetate was accomplished by placing the nitroglycerin in a vacuum desiccator for several days until constant weight was obtained. As a check on the purity, the refractive index of the nitroglycerin was determined at 15 degrees C. Duplicate determinations were 1.4740 and 1.4739. These values compare well to 1.474 at 15 degrees listed in Merck Index. The material was also assayed by the $TiCl_3$ method. The resulting average of several determinations was 99.0 per cent. The pure nitroglycerin was stored in a plastic beaker in a desiccator and amounts transferred as needed with the aid of a plastic dropper.

Four standard solutions were prepared by dissolving 0.3193, 0.4334, 0.5105, and 0.6142 grams of nitroglycerin in 50 ml of spectrograde benzene. Infrared measurements were taken at each of the three major absorption bands of nitroglycerin and the resulting data appear in Table 1. Plots of concentration versus absorbance showed adherence to Beer's law at each wavelength. Figure 1 shows the agreement of the 12 micron data to a straight line calculated and plotted by the least squares method.

Preparation of Samples. The double base propellant samples studied in this work contained nitroglycerin in the 20 to 30 per cent range. Four-gram samples were weighed out from the turnings produced when wafers of the solid propellant were drilled using a brace and bit. The weighed samples were placed in filter paper packets and the packets were placed in extraction thimbles in Soxhlet extractors. The samples were extracted with $MeCl_2$ and the resulting $MeCl_2$ solutions were evaporated to residues of constant weight. The last stages of the evaporation to constant weight were carried out by placing the samples in a vacuum system.

The constant-weight residues were dissolved in benzene and brought to volume in 100-ml volumetric flasks at 20 degrees C. These solutions were used to obtain to infrared data reported in this work. Portions of these solutions were withdrawn using hypodermic syringes and needles and were transferred to fixed thickness infrared cells with NaCl windows. The two cells used had thicknessses of 0.105 and 0.109 mm. The same cells were used for all determinations reported in this work, the 0.105 mm cell being used to contain the sample solution and the 0.109 mm cell being used to

TABLE 1.—Data for Standard Nitroglycerin Solutions.

Sample	Concentration (g/100 ml)	Absorbance 6.02μ	Absorbance 7.85μ	Absorbance 12.0μ
1	.6386	.627	.369	.225
2	.8668	.820	.493	.291
3	1.0210	.885	.560	.337
4	1.2284	1.000	.670	.399

contain the reference benzene solution. The instrument used for this work was a Perkin Elmer Model 21 infrared spectrophotometer.

Data Collection and Treatment. The method of obtaining infrared measurements involved recording only the peak being studied rather than the entire spectrum. To record the peak, a point of low absorbance (a valley) was chosen adjacent to the peak. The 100% adjust control on the instrument was set such that the pen was aligned at zero absorbance at the chosen starting wavelength. The peak was then recorded. This procedure was repeated several times for each peak in order to obtain a good average value of the absorbance for the solution at that wavelength. This method allows one to obtain several absorbance values in the time normally required to record an entire spectrum. The spectrum may be rapidly scanned to the area of interest and then a number of absorbance values may be recorded for the peak in question for any number of solutions.

The plot shown in Figure 1 served as a standard working curve for the 12 micron peak of nitroglycerin. The concentrations shown in Figure 1 are in terms of grams per 100 ml of solution although the actual standard solutions were prepared in 50 ml of benzene as described previously. This was done so that the absorbances and concentrations of the standards would correlate directly with those of the samples.

The absorbance of each sample at 12 microns was determined and the corresponding concentration of nitroglycerin in grams per 100 ml was read directly from the standard curve. The percentage of nitroglycerin in the sample was then calculated by this simple relationship.

$$\text{Per Cent Nitroglycerin} = \frac{\text{Concentration (g/100 ml)} \times 100}{\text{Sample Weight}}$$

RESULTS AND DISCUSSION

The data from the standard nitroglycerin solutions showed adherence to Beer's law at the 12 micron absorption band. This indicated that the 12 micron band should be suitable for the quantitative estimation of nitroglycerin in the concentration

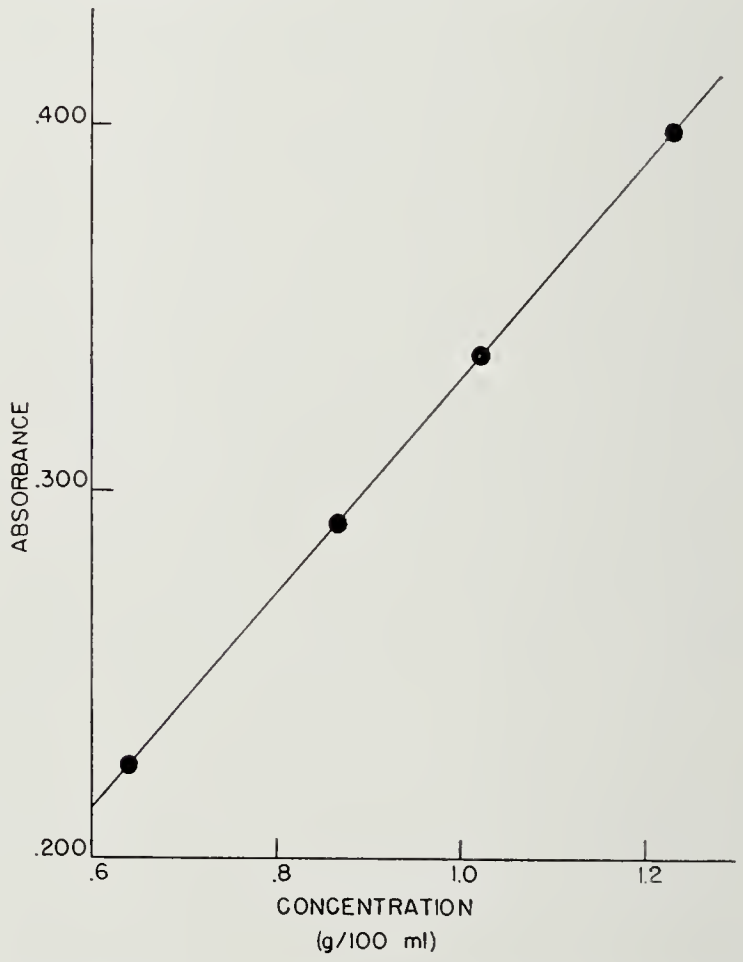

FIGURE 1. — Least Squares Evaluation of Data From Standard Nitroglycerin Solutions Using the 12 Micron Absorption Band.

TABLE 2.—Nitroglycerin Content of Double Base Propellant: Comparison of Infrared and $TiCl_3$ Methods.

Sample	A	Per Cent Nitroglycerin	
		IR Method	$TiCl_3$ Method
1	.306	22.9	23.1
2	.300	22.3	22.5
3	.301	22.4	22.8
4	.345	26.2	26.3
5	.322	24.2	24.1
6	.301	22.4	22.4
7	.358	27.2	26.5
8	.334	25.2	25.0
9	.338	25.6	25.0
10	.347	26.4	25.8
11	.310	23.2	22.3
12	.335	25.3	24.4
13	.325	24.5	24.5
14	.330	24.9	23.6
15	.314	23.5	22.4
16	.359	27.1	25.8
17	.338	25.6	24.8
18	.325	24.5	23.8
19	.310	23.2	23.2
20	.337	25.4	24.3
21	.317	23.8	22.6
22	.301	22.4	22.7
23	.330	24.9	23.8
24	.315	23.6	23.3
25	.324	24.4	24.6

range covered by the standard solutions.

The data obtained from 25 propellant samples appears in Table 2. The values for the per cent nitroglycerin found by the infrared method are compared to those found by the $TiCl_3$ method. Relatively good agreement exists between the two methods.

This work indicates that the 12 micron absorption band of nitroglycerin can be used for the quantitative estimation of nitroglycerin in solutions where the normally used absorption bands (6.02 and 7.85 microns) are masked by other infrared absorbing substances.

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OXYGEN AND PHOSPHATE METABOLISM OF BLUEGILL LIVER MITOCHONDRIA IN THE PRESENCE OF 2,4-DICHLOROPHENOXYACETIC ACID DERIVATIVES

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ABSTRACT. — The effects of the sodium salt, dimethylamine salt, the butyl ester, butoxy-ethanol ester, ethyl ester, isopropyl ester, isooctyl ester, propyleneglycolbutylether ester (PGBE), and 2-ethyl-hexyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D) on the oxygen and phosphate uptake by the bluegill liver mitochondria in the presence of succinate and alpha-ketoglutarate as substrates were investigated. High levels (3.7 — 6.4 $\times$ 10^{-4} g/ml of reaction media) of all the derivatives except the dimethylamine salt and the 2-ethyl-hexyl ester inhibited oxygen uptake in the presence of both substrates. Low levels (4.4 — 4.6 $\times$ 10^{-9} g/ml of reaction media) of the butyl ester and the isopropyl ester inhibited oxygen uptake. The butoxy-ethanol ester and the PGBE ester inhibited oxygen uptake and altered phosphate uptake. The sodium salt, the ethyl ester, and the isopropyl ester inhibited oxygen uptake but did not alter phosphate uptake. It appeared that the primary effect of 2,4-D derivatives was on oxygen metabolism. The butyl ester and isopropyl ester were the most effective in altering oxygen and phosphate uptake of the 2,4-D derivatives used in this study.

We observed in our laboratory that some formulations of 2,4-dichlorophenoxyacetic acid were more toxic to small bluegills (*Lepomis macrochirus*) than were other formulations (Hiltibran 1967a) and later found that some technical grade esters of 2,4-D were very toxic to small bluegills. The reports of Lawrence (1962) and Hughes and Davis (1963) confirmed and extended our observations.

Since we had initiated investigations to determine the effect of possible pollutants on the energy production by liver mitochondria from bluegill, the effect of nine derivatives of 2,4-D on the oxygen and phosphate uptake in the presence of succinic acid and alpha-ketoglutaric acid was estimated. The effect of 2,4-D derivatives on the hydrolysis of adenosinetriphosphate (ATP) by bluegill liver mitochondria was also investigated and has been reported (Hiltibran 1969).

MATERIALS AND METHODS

Native, wild bluegills were used in these experiments. The fish were held at 25° C. in the laboratory prior to use and all assays were conducted at that temperature.

The procedures for preparing the liver mitochondria, estimating oxygen and phosphate uptake, and estimating the nitrogen content of mitochondria have previously been reported (Hiltibran and Johnson 1965).

The bioassays to estimate the toxicity of 2,4-D and its derivatives to small bluegills were conducted as previously described (Hiltibran 1962).

Redistilled ethyl alcohol or acetone was used as the solvent for the water-insoluble derivatives of 2,4-D, and the various data have been corrected for the effects of the solvent. The data presented are the average values for at least three separate experiments, and all values are corrected for endogenous respiration. The concentrations of the derivatives in the reaction media are expressed as the grams of a derivative added to each milliliter of reaction medium.

Results

The toxicities of the technical grade or equivalent derivatives of 2,4-D to small bluegill are summarized in Table 1. The toxic levels of the technical grade derivatives agreed with the data reported by Hughes and Davis (1963), Lawrence (1962), and King and Penfound (1946). The isopropyl ester was slightly more toxic than the butyl ester, which was more toxic than the propyleneglycol butyl-ether ester. The sodium salt was the last toxic of the derivatives investigated.

Table 1.—Highest levels of the technical grade 2,4-D derivatives which did not cause death of small bluegills.

2,4-D Derivative	PPM
Butoxy-ethanol ester	6
Butyl ester	1
Ethyl ester	6
Isopropyl ester	<1
Isooctyl ester	40*
PGBE ester	2
2-ethyl-hexyl ester	40*
Dimethylamine salt	40*
Na salt	100

* Highest level of these derivatives used in these experiments.

Effects of 2,4-D Derivatives on the Succinic Oxidase

The effects of two concentrations of the nine derivatives of 2,4-D on the oxygen and phosphate uptake in the presence of succinic acid are summarized in Table 2. The dimethylamine salt and the 2-ethyl-hexyl ester of 2,4-D did not alter either oxygen or phosphate uptake. The sodium salt, the butoxy-ethanol ester, the ethyl ester inhibited oxygen uptake at the higher concentrations. While there was an increase in the inorganic phosphate content in the vessels containing the sodium salt and the ethyl ester, there was not an increase in the inorganic phosphate content in the vessels containing butoxy-ethanol ester. At the lower concentration neither the oxygen nor phosphate uptake was altered.

The butyl ester, the isopropyl ester, the isooctyl ester and the propyleneglycolbutyl-ether ester inhibited oxygen uptake at both concentrations. In the presence of the butyl ester and the PGBE ester there was not an increase in the phosphate content of the reaction media, but the phosphate content of the reaction media increased in the presence of the isopropyl and the isooctyl esters. (Lower concentrations of the butyl and isopropyl esters were used.)

Effects of 2,4-D Derivatives on Alpha-ketoglutarate Oxidase

The effects of the nine derivatives of 2,4-D on the oxygen and phosphate uptake by bluegill liver mitochondria in the presence of alpha-ketoglutarate as substrate are summarized in Table 3. The dimethylamine salt and the 2-ethyl-hexyl ester did not alter oxygen uptake at either concentration. The phosphate uptake in the preserve of the dimethylamine salt was not altered, but the phos-

TABLE 2.—Effects of 2,4-D on Succinic Oxidase.

Derivative	g/ml of Reaction Medium	Average Change in μl 0_2/hr/mgN	Average Change in μmoles PO_4/hr/mgN
Butoxy-ethanol ester	5.4×10^{-4}	(—) 50	(±) 7
	5.4×10^{-8}	(±) 34	(±) 9
Butyl ester	4.6×10^{-4}	(—) 44	(±) 12
	4.6×10^{-9}	(—) 16	(±) 8
Ethyl ester	4.8×10^{-4}	(—) 40	(+) 16
	4.8×10^{-8}	(±) 28	(±) 10
Isopropyl ester	4.4×10^{-4}	(—) 63	(+) 12
	4.4×10^{-9}	(—) 42	(+) 8
Isooctyl ester	5.6×10^{-4}	(—) 33	(+) 9
	5.6×10^{-7}	(—) 42	(±) 7
PGBE ester	6.4×10^{-4}	(—) 25	(±) 6
	6.4×10^{-8}	(—) 38	(±) 8
2-ethyl-hexyl ester	5.6×10^{-8}	(±) 25	(±) 10
	5.6×10^{-7}	(±) 23	(±) 11
Dimethylamine salt	4.4×10^{-4}	(±) 16	(±) 10
	4.4×10^{-8}	(±) 13	(±) 9
Na salt	3.7×10^{-4}	(—) 79	(+) 20
	3.7×10^{-8}	(±) 50	(±) 9

phate uptake in the presence of the 2-ethyl-hexyl ester was altered.

At the higher concentration, the sodium salt, the butoxyethanol ester, the ethyl ester, the isooctyl ester and the PGBE ester inhibited oxygen uptake but did not alter oxygen uptake at the lower concentration. At the higher concentration, in the presence of sodium salt and ethyl ester, there was an increase in the phosphate content of the reaction medium, but in the presence of the butoxyethanol ester, the isooctyl ester and the PGBE ester, there was not an increase in the phosphate content of the reaction medium. At the lower concentration only the sodium salt had an effect on phosphate uptake.

The butyl ester and the isopropyl ester inhibited oxygen uptake at both concentrations. At the higher concentration of the butyl ester there was a decrease in the phosphate content of the reaction medium, whereas at the same concentration of the isopropyl ester there was an increase in the phosphate content of the reaction media. At the lower concentration there was not an increase in the phosphate content of the reaction media.

DISCUSSION

The above data indicate that various derivatives of 2,4-D can alter the oxygen and phosphate uptake by the bluegill liver mitochondria.

TABLE 3.—Effects of 2,4-D on Alpha-ketogultarate Oxidase.

Derivative	g/ml of Reaction Medium	Average Change in μl O_2/hr/mgN	Average Change in μmoles PO_4/hr/mgN
Butoxy-ethanol ester	5.4×10^{-4}	(—) 110	(±) 13
	5.4×10^{-8}	(±) 46	(±) 13
Butyl ester	4.6×10^{-4}	(—) 81	(—) 25
	4.6×10^{-8}	(—) 34	(±) 9
Ethyl ester	4.8×10^{-4}	(—) 105	(+) 27
	4.8×10^{-8}	(±) 39	(±) 12
Isopropyl ester	4.4×10^{-4}	(—) 135	(+) 29
	4.4×10^{-8}	(—) 34	(±) 23
Isooctyl ester	5.6×10^{-4}	(—) 37	(±) 17
	5.6×10^{-8}	(±) 83	(±) 9
PGBE ester	6.4×10^{-4}	(—) 62	(±) 21
	6.4×10^{-8}	(±) 85	(±) 19
2-ethyl-hexyl ester	5.6×10^{-4}	(±) 34	(—) 16
	5.6×10^{-8}	(±) 40	(±) 33
Dimethylamine salt	4.4×10^{-4}	(±) 74	(±) 19
	4.4×10^{-8}	(±) 30	(±) 17
Na salt	3.7×10^{-4}	(—) 118	(+) 33
	3.7×10^{-8}	(±) 5	(+) 4

The dimethylamine salt and the 2-ethyl-hexyl ester did not alter oxygen uptake in the presence of either substrate, however, the phosphate uptake was increased by the 2-ethyl-hexyl ester in the presence of alpha-ketoglutarate, but not in the presence of succinate.

The sodium salt, the butoxyethanol ester, and the ethyl ester appeared to have similar effects on both oxidase complexes, except that at the low concentration the sodium salt increased the phosphate content of the reaction media in the presence of alpha-ketoglutarate. The isooctyl and the PGBE esters inhibited oxygen uptake at both concentrations in the presence of succinate, but inhibited oxygen uptake in the presence of alpha-ketoglutarate only at the high concentration. The PGBE ester did not increase the phosphate content of the reaction media in the presence of either substrate, but the isooctyl ester increased the phosphate content of the reaction media in the presence of succinate only at the higher level.

The butyl ester and the isopropyl ester were the only 2,4-D derivatives which inhibited oxygen uptake in the presence of both substrates. However, their effects on phosphate uptake were different. In the presence of succinate the phosphate content was not increased in the presence of the butyl ester, whereas at the high concentration a decrease in the phosphate content occurred in the presence of alpha-ketoglutarate. In the

presence of the isopropyl ester, on the other hand, phosphate content of the reaction media increased except at low levels in the presence of alpha-ketoglutarate.

The effect of several enzyme inhibitors on the oxygen and phosphate uptake in the presence of the two substrates has been reported (Hiltibran 1965, 1967*b*). For example, agents such as sodium azide, sodium cyanide inhibited the flow of electrons from the substrate to oxygen and the phosphate content of the reaction media increased, which indicated that inorganic phosphate was released from tissue phosphate. Therefore, it would appear that the primary effect of the sodium salt, the ethyl ester, and the isopropyl ester of 2,4-D would be the inhibition of oxygen uptake with little or no effect on phosphate uptake, as indicated by the increase in the phosphate content of the reaction media.

In the presence of high levels of butoxyethanol ester, butyl ester, and PGBE ester, oxygen uptake was inhibited but there was not an increase in the phosphate content of the reaction media which indicated that the three esters had some effect on phosphate uptake. At the lower concentrations only, the butyl ester and the PGBE ester, in the presence of succinate, inhibited oxygen uptake but there was not an increase in the phosphate content of the reaction media. Of these esters, only the butyl ester inhibited oxygen uptake at low levels in the presence of alpha-ketoglutarate; at high levels the effect of the butyl ester on phosphate metabolism was more pronounced.

The effects of the isooctyl ester on phosphate uptake of the two complexes are different; also, in the presence of succinate as substrate, low levels of the isooctyl ester inhibited oxygen uptake.

The butyl and the isopropyl esters inhibited oxygen uptake at the lowest concentrations (4.4 and 4.6 $\times$ 10^{-9} g) and since the effect of these two esters on the phosphate uptake was different in the presence of succinate as substrate, this small difference in the effect on phosphate metabolism would indicate that the isopropyl ester could disrupt energy production by bluegill liver mitochondria more than the butyl ester. The isopropyl ester was found to increase the hydrolysis of ATP at a lower level than the butyl ester (Hiltibran 1969).

The butyl ester was the next most effective ester, and because of its effect on the conversion of inorganic phosphate to tissue phosphate, it would appear to disrupt the energy production to a lesser extent. The PGBE ester would appear to disrupt energy production less than the isopropyl ester or butyl ester. The butoxyethanol ester and the ethyl ester disrupted energy production to a lesser extent than the above three esters, but more than the isooctyl ester and the sodium salt. The dimethylamine salt and the 2-ethyl-hexyl ester did not greatly alter energy production. The decreasing order of effectiveness of these 2,4-D derivatives on the energy production of the bluegill liver mitochondria correlates with the decreasing order of toxic values for these derivatives. The data also suggect, however, that most of the derivatives used in this study can be detrimental to bluegills, as at high levels all except the dimethylamine salt were found to have some effect on energy production.

The data suggest that the toxicity of these derivatives to bluegills may be related to the effects of these derivatives on the production of energy by the bluegill liver mitochondria.

At high levels of some of the 2,4-D derivatives in the presence of both substrates oxygen uptake was inhibited, which indicated an interference at some point common to the oxidation of both substrates. The data were reported on the basis of the grams of the derivatives per milliliter of reaction medium, and indicated that the various esters or salts of 2,4-D had specific effects on the oxygen and phosphate utilization by the bluegill liver mitochondria. It appeared, therefore, that the ester or salt derivatives conferred on the 2,4-D molecule a specificity of action toward the bluegill liver mitochondria enzyme systems.

Most of the effects of 2,4-D on plant systems are attributed only to 2,4-D (Audus 1964). The data summarized by Bonner and Bandurski (1952), Switzer (1957), and Wedding and Black (1961) indicated that 2,4-D uncoupled the phosphorylation from the oxidation of substrates by plant tissues. Brody (1952) reported that 2,4-D uncoupled the phosphorylation from substrate oxidation in rat liver mitochondria systems. Data presented above of the effects of the 2,4-D derivatives on the bluegill liver mitochondrial enzyme complexes are in contrast to the effects described for plant and animal systems.

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COMPUTED SOIL MOISTURE PATTERNS IN AND AROUND THE PRAIRIE PENINSULA DURING THE GREAT DROUGHT OF 1933-34

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ABSTRACT. — Soil moisture patterns were derived for a classical drought in and around the Prairie Peninsula.

Weather data was collected from the first order weather stations within the midwest, and using Thornthwaite's water balance computations, soil moisture patterns for the drought years 1933-34 were computed and plotted on maps. Spring soil moisture storage and moisture deficits were computed using an available soil moisture holding capacity of 14 inches.

Iso-lines of available soil moisture and moisture deficits for the spring of 1934 exhibit patterns which are generally peninsular, some of which closely outline the area that Transeau designated as the Prairie Peninsula.

Both the Prairie Peninsula (Figure 1) and the Great Plains were predominantly grasslands in pre-settlement times. The Prairie Peninsula, however, included forests growing on a variety of upland sites as well as lowland sites while in the Great Plains, forests were restricted largely to riparian strips and certain other highly localized environments.

The climate of the Great Plains is such that no one questions its ability to exclude forests from soils which depend on precipitation for their total water supply. No general agreement exists, however, as to why the Prairie Peninsula had a pre-settlement vegetation dominated by grass while the region on its northeast flank was largely forested. Classical climatic comparisons indicate that precipitation in both regions is high enough to permit tree growth. Transeau (1935), however, showed that the Prairie Peninsula has a lower mean P/E ratio than adjacent forested regions. The moisture index of Thornthwaite and Mather (1955) also has lower values in the Prairie Peninsula than in adjacent forested regions. While both of these correlations suggest a less favorable mean water balance in the Prairie Peninsula, they do *not* indicate that this average condition is actually unfavorable or lethal to forests. Nor could these studies indicate this since they did not involve the evaluation of an environmental factor directly influencing the internal water balance of plants such as available soil moisture.

Extreme climatic conditions in the Prairie Peninsula, however, apparently can instigate conditions lethal to trees. Transeau (1935) noted the death of thousands of oaks bordering the prairies in Illinois by the close of the 1913-14 drought. McComb and Loomis (1944) reported the death of, or severe damage to hundreds of thousands of adapted trees

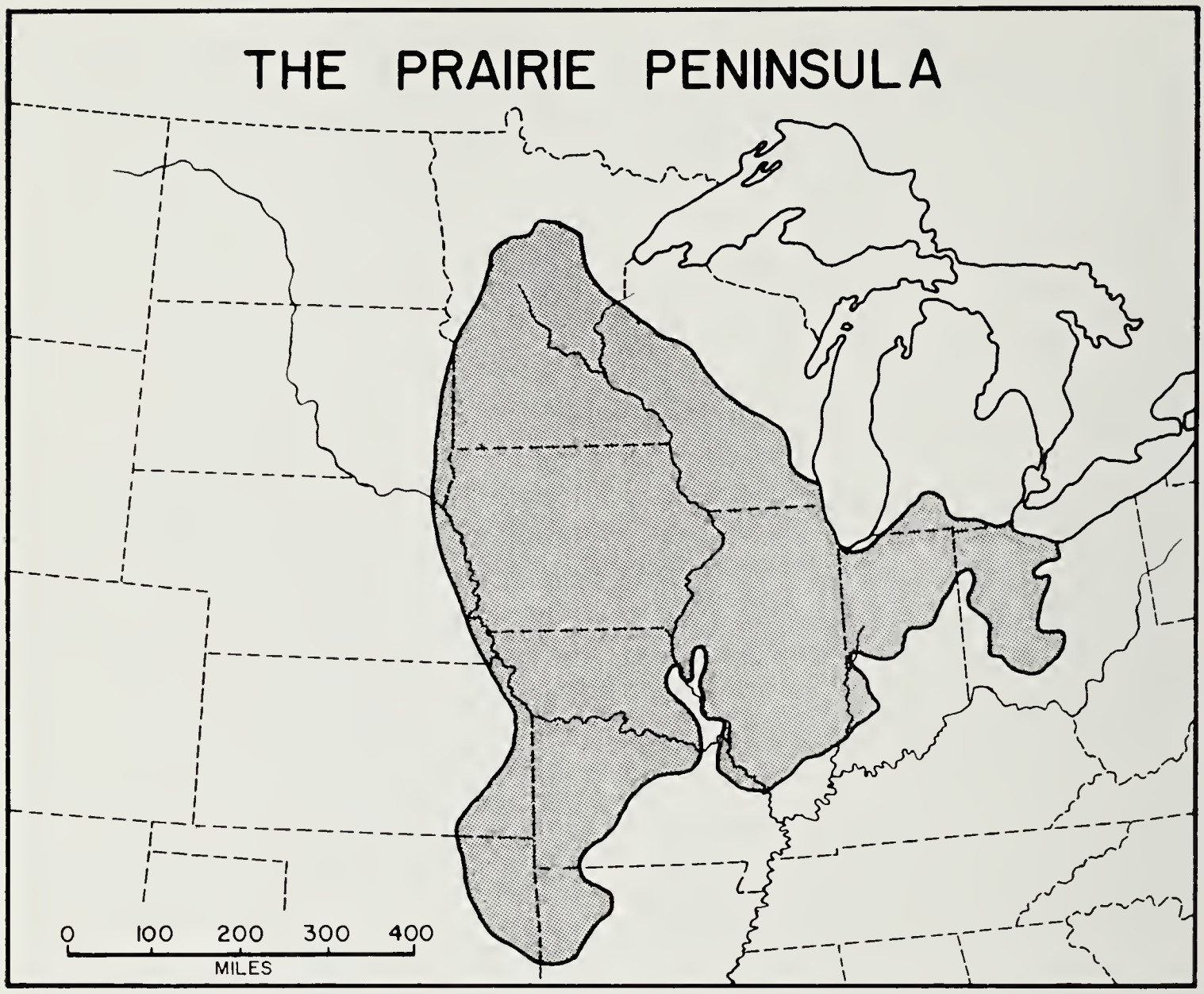

FIGURE 1. — The Prairie Peninsula.

on suitable or even protected sites during the droughts of the 1930's in Iowa. They also observed that there was no such wholesale damage to the woodlands outside the grassland. Shelford (1963) states that the oak-basswood forests suffered the most damage during this time. In eastern Nebraska, for instance, 80 percent of the red oak and basswood within 25 kilometers of the Missouri River died during the drought years of the early 1930's. Shelford also gives an account of events in Trelease Woods near Urbana, Illinois during the dry spring of 1934. Many forest trees began to lose their leaves in July of that year and were almost completely defoliated by August. Several trees died.

Blake (1935) found that during the years of drought, practically the entire available soil water supply was used. He stated that during these years, tree seedlings frequently appeared in the priarie but few became established.

Was there something unique about these tree-killing droughts of 1913-14 and the early 1930's? Drought obviously does not always kill trees nor does its frequent occurrence always discriminate against trees in favor of grass. Forested areas having Mediterrean-type climates, such as those near the Pacific Ocean, are characterized by severe summer droughts.

Daubenmire (1947) suggests that the major difference in the effect these droughts have on the forests as compared to the droughts of the Prairie Peninsula lies in the timing of the droughts. Those in the Pacific area take place in late summer after the trees have become dormant. Thornthwaite and Carter (1958) mention that droughts in the Prairie Peninsula generally take place in April and May. Kozlowski (1962) reports that this is the bulk of the growing season for many of the trees found in this area. He quotes Johnston (1941) as stating that three species of oak in Missouri complete practically all of their height growth in only 19 days.

Borchert (1951) concluded that the Prairie Peninsula has had a climate more like that of the steppe than the eastern forests during most winters and summers of major drought years. The question arises as to whether one facet of this similarity is more important than others as concerns detrimental effects on trees.

The soils in steppe climates are characterized by having B horizons which are below field capacity and C horizons which are perpetually dry. The C horizons remain dry primarily because the low fall and winter precipitation is not sufficient to recharge the soils with moisture to that depth.

Since seasonal water deficits in the Prairie Peninsula typically occur in spring, there is a chance that some of them may be associated with dry winters. The possibility of a dry winter followed by a dry spring which coincides with the growing season of oaks suggests a more detrimental soil moisture regime to these trees than either climatic facet taken alone.

Based on this line of reasoning, the present study was designed to determine the geographic distribution of calculated soil moisture patterns in the North Central States during the spring of 1934. In 1934, trees were prematurely defoliated if not killed in Trelease Woods. Evidence that the preceding dry fall and winter may have contributed to the severity of the 1934 drought is given by Holcomb (1934). He states that in Illinois "during 1933, subsoil moisture was very deficient during the last several months of the year, with reports increasing of wells and springs going dry."

Procedures

The computational tables of Thornthwaite and Mather (1957) were used to calculate soil moisture storage and deficits. An available soil moisture-holding capacity of 14 inches was used in the computations.

Iso-lines of storage (available water) and deficits were determined by mathematical interpolations between plotted values.

Results and Discussion

Iso-lines of soil moisture storage at the end of April, 1934 (see Figure 2) roughly parallel the humid boundaries of the Prairie Peninsula. Low storage values extending eastward from the Great Plains are conspicuously concentrated within the Prairie Peninsula. Small islands of non-conformity are presumably due to local weather patterns for this particular drought period.

In some areas of Indiana and Illinois, soils were completely recharged by the end of March but due to a dry April were down below field capacity again by the end of that month. These soils, therefore, probably did not have dry subsoils like the soils in

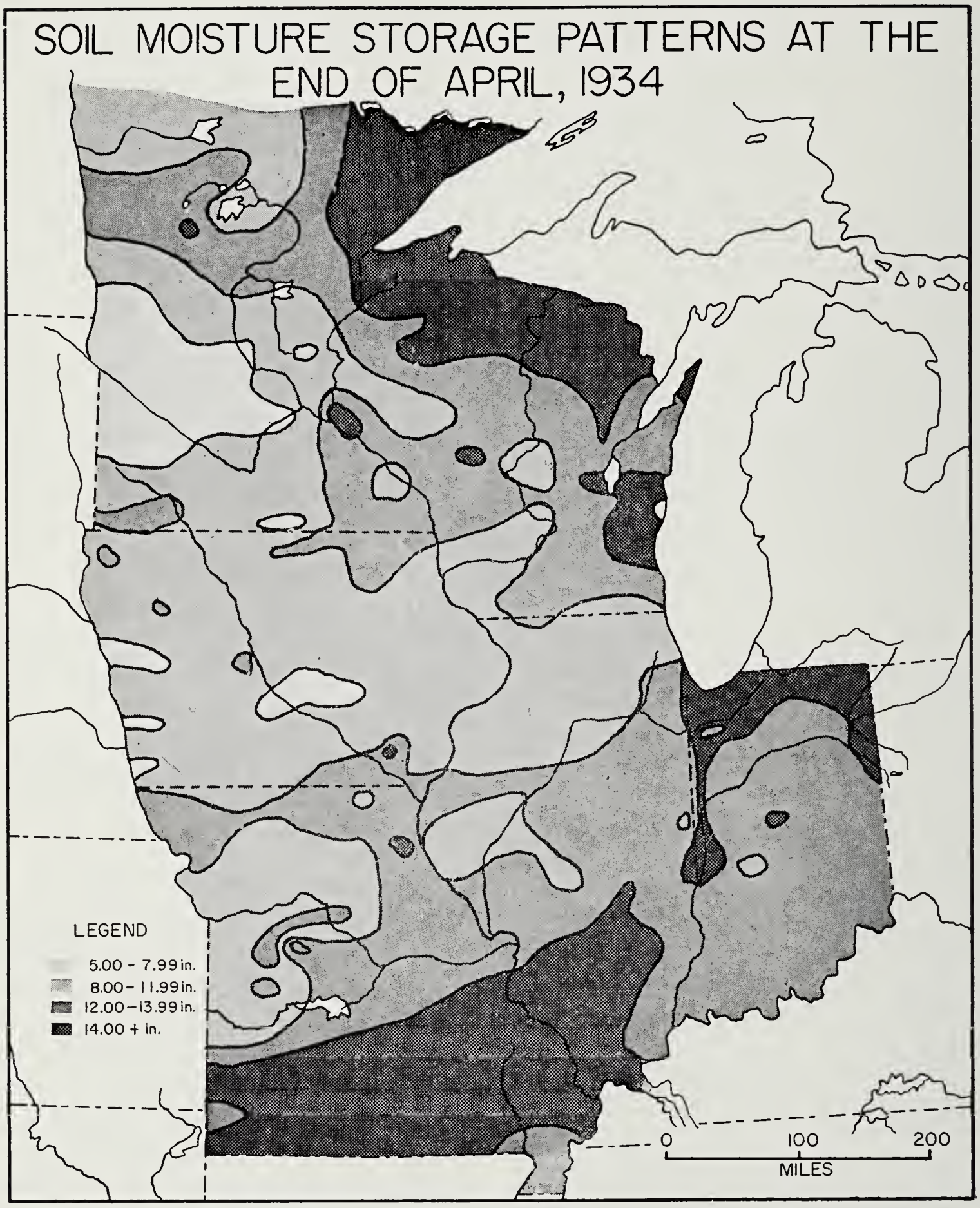

FIGURE 2. — Soil Moisture Storage Patterns At The End Of April, 1934.

other areas which were not completely recharged at any time during the 1933-34 hydrological year. Therefore the map in Figure 3 was drawn to separate the areas where complete recharge did occur from those where it did not occur. The resulting pattern outlines the Prairie Peninsula quite well except for northern Minnesota.

Since soil recharges with moisture from the surface downward, the soils which were not completely recharged would have had dry subsoils during

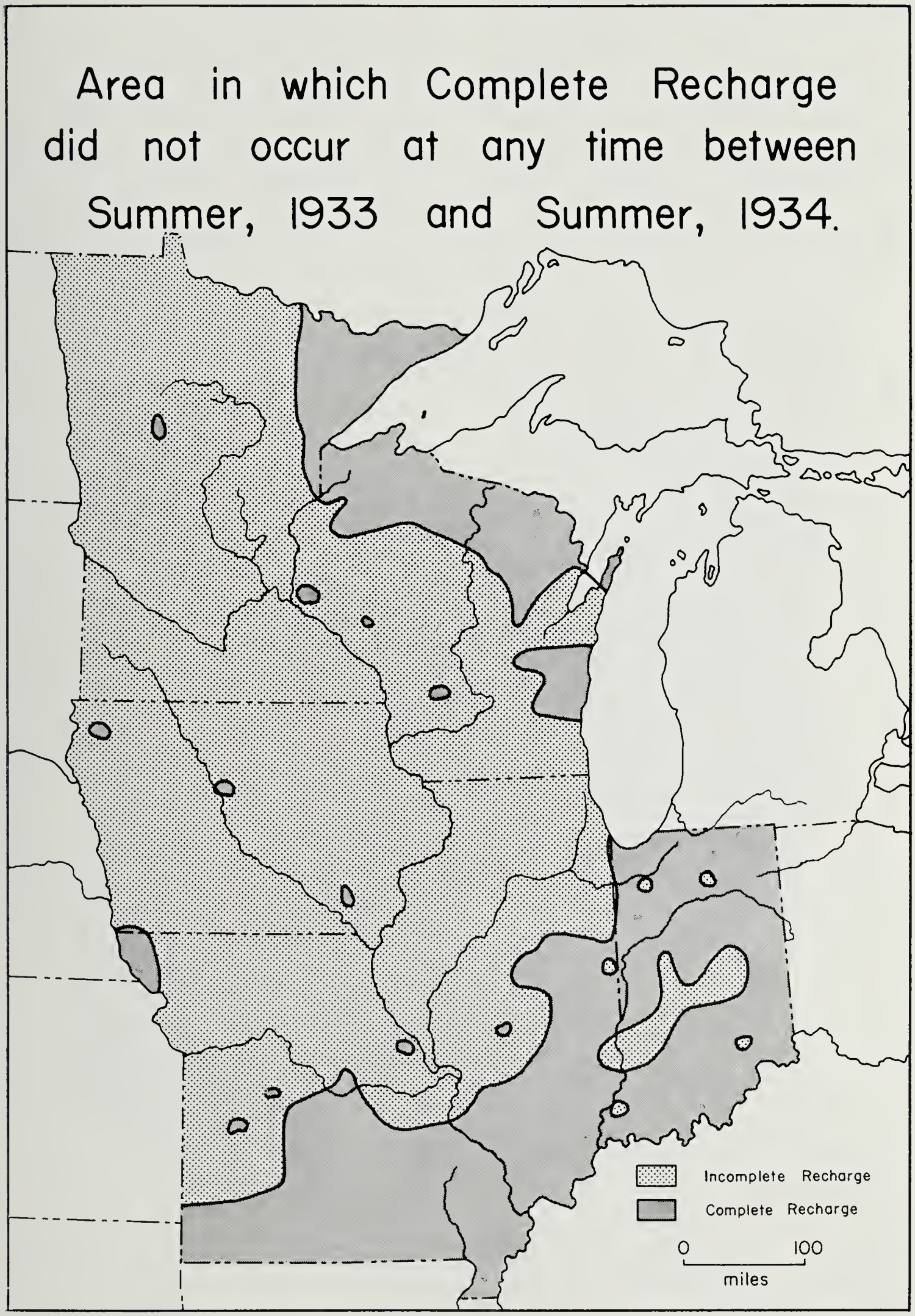
Area in which Complete Recharge did not occur at any time between Summer, 1933 and Summer, 1934.
Incomplete Recharge
Complete Recharge
0
100
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FIGURE 3. — Area In Which Complete Recharge Did Not Occur At Any Time Between Summer, 1933 and Summer, 1934.

the months of April and May. This type of dryness in conjunction with the dry May could have had a more detrimental effect on deep-rooted trees than on grasses. According to Thompson (1957) "rain water may not penetrate very deep into the soil, but the wetting of the surface soil supplies the needs of grasses where trees would not survive."

By the end of May, 1934 water deficits were distinctly concentrated in the Prairie Peninsula (Figure 4) where normally there is none at that

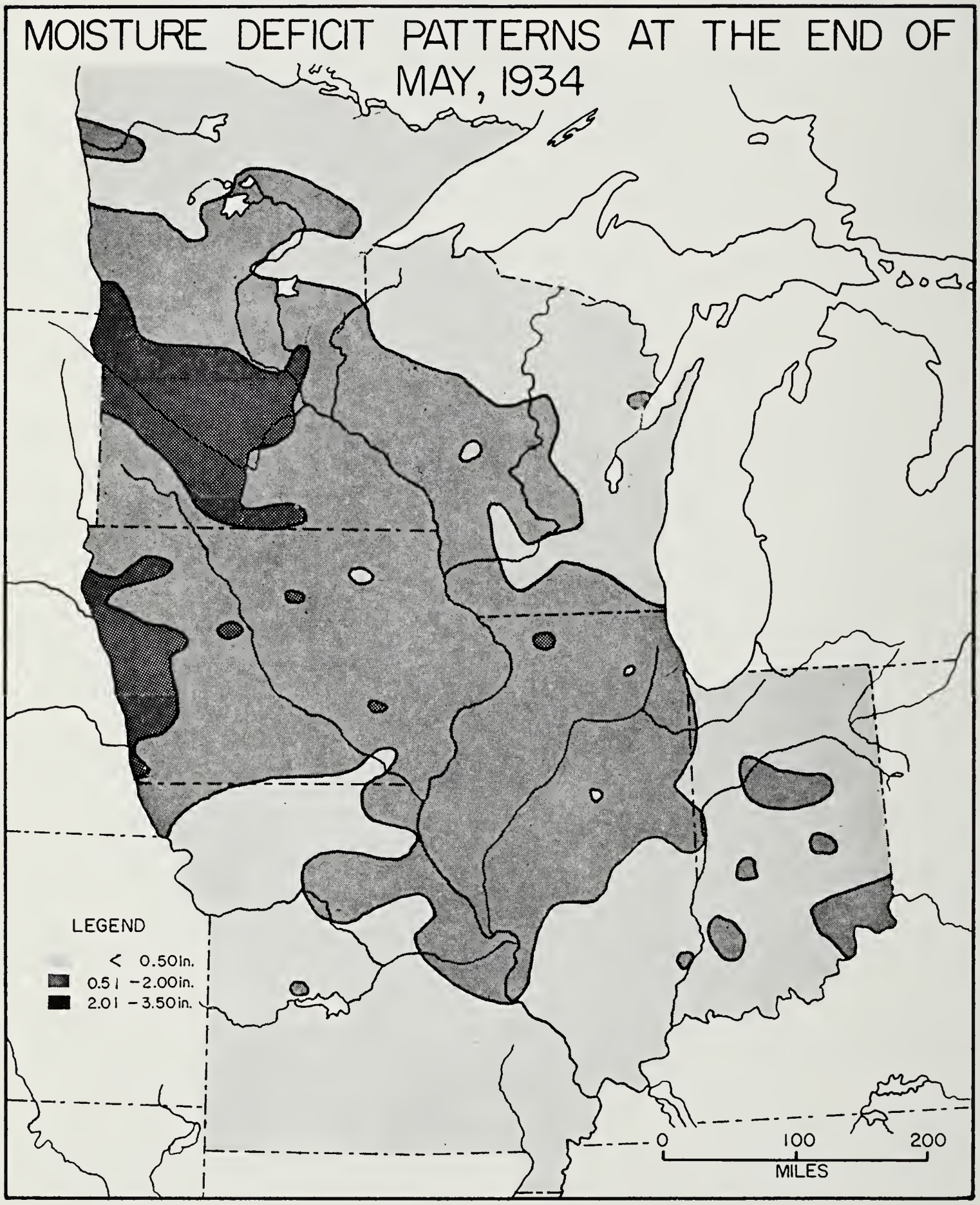

FIGURE 4. — Moisture Deficit Patterns At The End Of May, 1934.

time of year. In addition to the high deficits, *per se*, this extreme variation from normal values may have been important, too, since in areas which normally receive such high deficits, the vegetation seems to be composed of more xerophytic species or have more xerophytic characteristics (Daubenmire, 1947).

Conclusions

Computed soil moisture patterns during the drought of 1933-34 indicate that the soil moisture regime in the Prairie Peninsula was similar to the normal regime in steppe areas such as the Great Plains. It is suggested that this type of regime coupled with abnormally high water deficits in May could have been instrumental in the deaths of many trees. It is further suggested that other drought periods exhibiting climatic similarities to steppe areas may also have resulted in similar soil moisture regimes.

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DISCRETE CHARACTERIZATION OF THE SUB-FRACTIONS OF PORCINE HAPTOGLOBIN (BAND 5)

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ABSTRACT. — The electrophoretic pattern for porcine haptoglobin (band 5 or Hp5) as devloped with pig hemoglobin and observed after starch gel electrophoresis, consists of two bands of nearly equal width and intensity; while the pattern developed with chicken hemoglobin consists of three bands (Hp5-F, Hp5-M and Hp5-S), of which Hp5-F and Hp5-M are equally narrow and intense, and Hp5-S is approximately four times as wide and intense as the others.

A variety of electrophoretic patterns of Hp5-pig hemoglobin complexes has been detected with the use of chicken hemoglobin. These uniformly varying patterns are dependent on the quantity of pig hemoglobin complexed with Hp5 prior to the addition of chicken hemoglobin.

In pig serum after starch gel electrophoresis, three benzidine staining bands (Hp1, Hp2 and Hp3) were initially called haptoglobins (Kristjansson, 1961), but further work has shown that they are hemebinding proteins and have subsequently been named hemopexins (Imlah, 1965; Graetzer *et al.*, 1965; Schröffel, 1966; Baker, 1967; Grunder, 1968). Similarly, a fourth band Hp0 (Hesselholt, 1963), and a fifth band Hp4 (Baker, 1967), belong to the hemopexin system in pigs.

If pig serum contained no pig hemoglobin, a sixth band named Hp5 (Kristjansson, 1961), on combination with chicken hemoglobin separated into three fractions on starch gels after electrophoresis and benzidine staining (Hesselholt, 1963; Mittal, 1966). Evidence has been reported that only the benzidine staining globins in this band 5 (Hp5) region are the 'true' haptoglobins (Hesselholt, 1963; Imlah, 1965; Grunder, 1968).

This report describes discrete characterization of the sub-fractions of the 'true' porcine haptoglobins (Hp5). In addition, evidence is presented showing variation in the electrophoretic mobility of these sub-fractions when combined with various amounts of porcine hemoglobin.

MATERIALS AND METHODS

Chicken hemoglobin solution (CHB) prepared from the fresh blood of a White Leghorn hen containing approximately 7% (w/v) hemoglobin, and pig hemoglobin solution (PHB) prepared from the fresh blood of a Yorkshire pig containing approximately 11.5% (w/v) hemoglobin were used (Hesselholt, 1963). Nonlysed pig serum was prepared from the blood of an eight-month-old Duroc boar, and was used in 0.25 ml. aliquots.

Ten serial dilutions of PHB were prepared with distilled water and coded W (undiluted), W^1 (1 in 2), W^2 (1 in

4), ..., W^9 (1 in 512) respectively. CHB was used in the dilution of 1 in 2.

Experiment 1. Eleven 0.25 ml. aliquots of pig serum were placed in tubes 1 to 11. One drop (22 drops = 1 ml.) of a dilution of PHB (W to W^9 respectively) was added to a corresponding tube (1 to 10 respectively) containing pig serum. No PHB was added to tube 11 (serum control). A 0.25 ml. volume of distilled water was placed in tube 12, and one drop of PHB (W) was added to it (PHB control). The 12 tubes were incubated at 37°C. for 30 min. with periodic agitation, and stored at —20°C., until thawed and examined by stark gel electrophoresis.

Experiment 2. To first 10 aliquots prepared as in experiment 1 (addition of PHB and incubation), a drop of CHB was added to each tube (1 to 10), and they were again incubated. Six controls were included in the test: (11) serum + PHB; (12) serum + CHB; (13) serum; (14) distilled water + PHB + CHB; (15) distilled water + PHB; and (16) distilled water + CHB. The volume of serum or water was 0.25 ml., and one drop of respective hemoglobin (PHB undiluted; CHB dilution 1 in 2) was added in each case. Controls were incubated at the same time as the test samples, and stored at —20°C.

Samples in both experiments were examined by starch gel electrophoresis at 4°C., followed by benzidine staining (Smithies, 1959; Kristjansson, 1961; Hesselholt, 1963; Mittal, 1965).

Results and Discussion

Jayle and Moretti (1962) reported on the important role of hemoglobin in the haptoglobin metabolism of the human. Also much evidence has been presented on the characteristics of the human haptoglobins, as well as the variations in the electrophoretic mobilities of their complexes with different amounts of hemoglobin (Laurell, 1959; Smithies *et al.*, 1962; Nance and Smithies, 1963). This study focused on porcine haptoglobin and the electrophoretic mobilities of its many intermediate complexes formed with different amounts of the pig hemoglobin. These complexes have been further analysed using a heterologous hemoglobin (chicken), which characteristically separates pig haptoglobin into three sub-fractions.

In the first experiment, a constant volume of various dilutions of PHB was added to a constant volume of pig serum (constant amount of pig haptoglobin). The complexes formed between the constant amount of Hp5 and the higher amounts of pig hemoglobin (lowest five dilutions of PHB, samples 1 to 5) appear (Figure 1) as a single densely staining band (D), while the complexes between the same amount of Hp5 and lower amounts of pig hemoglobin (higher dilutions of PHB, samples 6 to 10) appear as two closely spaced and less intense bands (C and D). Serum control presented two extremely faint bands, perhaps due to extremely minute amounts of endogenous PHB in pig serum.

When tested by itself (PHB control, 12 in Figure 1), PHB separated into two bands referred to as free hemoglobin 1 (fast moving) and free hemoglobin 2 (slow moving) by Kristjansson (1961), here symbolized as pig Hb 1 and pig Hb 2 respectively (A and B bands in Figure 1; the two bands are not clear in Figure 1, they can be seen in Figure 2, samples 1, 2, 3, 11, 14 and 15). The pig Hb 2 band is wider than pig Hb 1, and both migrated faster than pig Hp5 fractions (C and D, Figure 1).

In the second experiment, again a constant volume of the various dilutions of PHB was added to a constant volume of pig serum first. Thus variable amounts of pig hemoglobin were allowed to combine with a constant amount of pig haptoglobin. Then a constant volume of a 1 in 2 dilution

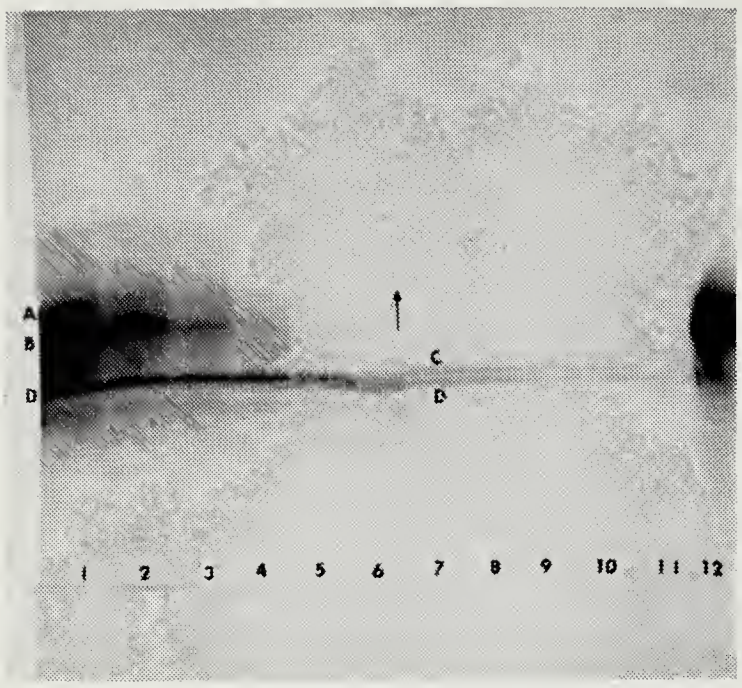

FIGURE 1. — Starch gel electrophoresis of boar serum. Benzidine stain. Arrow indicates the direction of migration of proteins in the gel. 1 to 10-serum aliquots to which serial dilutions of pig hemoglobin (W, W^1.....W^9 respectively)were added. Volumes of serum and pig hemoglobin solution (PHB) were equivalent for all samples. 11-Serum control (no hemoglobin added); 12-PHB control (no serum included); A-Band of pig Hb1 (also Figure 2); B-Band of pig Hb2 (also Figure 2); C-Band of pig Hp5 (fast moving); D-Band of pig Hp5 (slow moving).

of CHB (thus a constant amount) was added to each of the above mixtures.

Pig Hp5 combined with CHB alone (12 in Figure 2), separated into three fractions or bands designated Hp5-F, Hp5-M and Hp5-S (D, E and F bands respectively in Figure 2) in decreasing order of migration rate in starch gel. The Hp5-F and Hp5-M bands were narrow, and the Hp5-S band was twice as wide and intense as the others. Band C of experiment 1 (Figure 1) did not appear in the starch gel of experiment 2.

In the samples containing variable amounts of PHB, a serial change was observed in the patterns of Hp5 bands corresponding to the decreasing amounts of PHB (1 to 10, Figure 2). Hp5 was apparently saturated with PHB in samples 1 to 5 since only one band Hp5-F appeared with W to W^4, but this band decreased in width and intensity in W^5 to W^9 (6 to 10, Figure 2) as the other sub-fractions Hp5-M and Hp5-S began to appear. Hp5-M appeared in W^5, was of maximum intensity in W^6 and maintained width and intensity comparable to Hp5-F in W^7 to W^9 (8 to 10, Figure 2). Hp5-S appeared in W^6, gradually increased, and was widest and most intense in W^8 and W^9. Sample 5 (Figure 2) indicates the threshold dilution (W^4, PHB dilution 1 in 16) below which an amount of Hp5 is apparently left *unbound*, and thus is available for binding with CHB added later to develop the three bands characteristic of pig Hp5 bound with CHB (12 in Figure 2).

When tested by itself, CHB separated into four fractions (CHB control, 16 in Figure 2) designated chicken Hb 1, Hb 2, Hb 3 and Hb 4 (G, H, I and J respectively in Figure 2) in decreasing order of migration in starch gel. Bands of chicken Hb 1, Hb 2 and Hb 3 were of identical width, the Hb 4 band being wider.

When PHB and CHB were mixed, they migrated separately, apparently unaffected by the presence of the other (14, Figure 2), and the fractions of each migrated at the same rates as when tested independently (compare control 14 with 15 and 16, Figure 2). Control 13 (serum control) produced no detectable band. The pattern for serum + PHB control (11 in Figure 2) appeared similar to that of sample 1 of experiment 1 (1 in Figure 1), as expected.

In conclusion, for pig Hp5, two discrete sub-fractions have been observed with the use of homologous

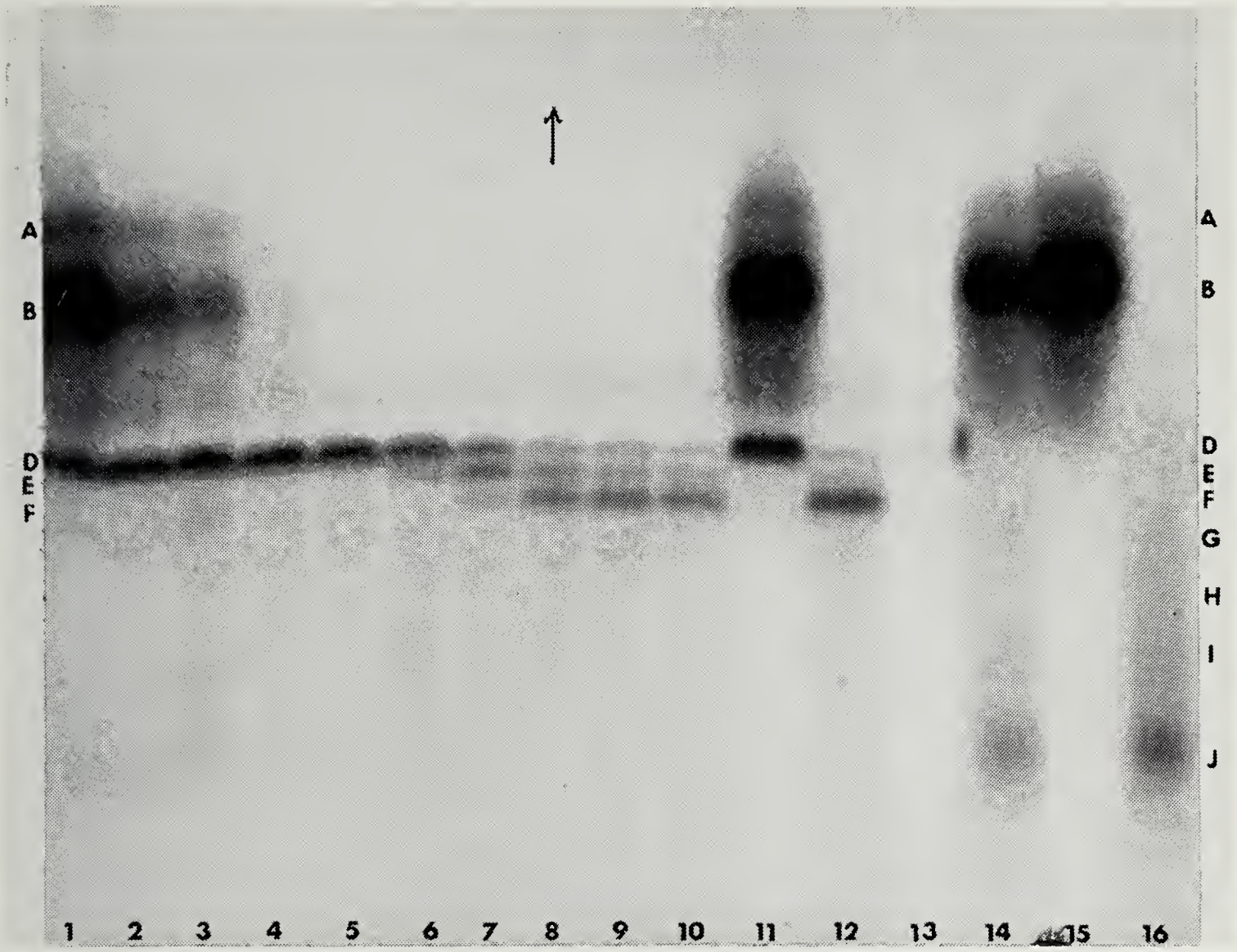

FIGURE 2. — Starch gel electrophoresis of boar serum. Benzidine stain. Arrow indicates the direction of migration of proteins in the gel. 1 to 10-serum aliquots to which serial dilutions of PHB (W,W^1.....W^9, respectively) were added first, and then chicken hemoglobin (dil 1 in 6) was added; 11 — serum + PHB control; 12 — serum + chicken hemoblobin (CHB) control; 13 — serum control; 14 — PHB + CHB control; 15 — PHB control; 16 — CHB control. A — Band of pig Hb1; B — Band of pig Hb2; D — Band of pig Hp5-F; E — Band of pig Hp5-M; F — Band of pig Hp5-S; G — Band of chicken Hb1; H — Band of chicken Hb2; I — Band of chicken Hb3; J — Band of chicken Hb4.

hemoglobin while three discrete sub-fractions have been detected with the use of heterologous (chicken) hemoglobin. The wide variety of patterns of pig Hp5-hemoglobin complexes observed in this study appeared dependent on the amount of PHB with which pig Hp5 was complexed. As the amount of PHB decreased so that it was insufficient for complex formation with all the molecules of Hp5 sub-fractions, the remaining unbound molecules formed complexes with CHB which was added later, thus gradually producing the characteristic three band pattern (7 to 10, Figure 2).

ACKNOWLEDGMENTS

The authors express their profound gratitude to Dr. B. A. Rasmusen and Dr. A. V. Nalbandov, University of Illinois, Urbana, and Dr. A. Nisonoff, University of Illinois Medical Center, Chicago, for their most valuable guidance and help without which this study would not have been possible.

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RESULTS OF A FIELD TEST TO CONTROL STRIPED SKUNKS WITH DIETHYLSTILBESTROL

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ABSTRACT. — Data concerning movements of striped skunks (*Mephitis mephitis*) in the wild indicated that the movements of nine diethylstilbestrol (DES)-treated skunks were not restricted after treatment. In a field trial, more than 82 percent of 9,000 DES-tallow baits distributed annually, in 1965 and in 1966, on a 46,000-acre area in Carroll County, Illinois, were taken within 10 days after placement. Samples of skunks collected from the treated area and a control area indicated no marked changes in numbers of skunks from 1965 to 1966. Differences in number of placental scars per female and in size of gonads of both sexes between skunks from the treated area and those from the control area were not significant ($P>0.05$). The results of the field test did not show that the DES treatment resulted in a significant reduction in skunk productivity.

This paper presents the results of preliminary field tests to suppress productivity in striped skunks in Carroll County, Illinois using the synthetic estrogen diethylstilbestrol (DES). Although further studies are needed to adequately demonstrate the value of this technique, these preliminary results are presented because it was not possible to continue this study. DES was selected because of its reported antifertility effects in other mammals (Greenwald 1957, Hill and Pierson 1958, Balser 1964, and Linhart and Enders 1964), and because it is readily available and inexpensive.

Thanks are due Dr. E. R. Shepard, The Eli Lilly Company, for assaying a sample of DES baits used in the field tests; K. P. Dauphin for assistance in the field; and Dr. B. J. Verts, Dr. P. J. Dziuk, and Mrs. Helen C. Schultz for editorial suggestions.

METHODS

Reproduction of Adult Females

Four adult females captured in March, 1966, in Carroll County, were fed cat food containing DES. Two each received 25 mg/day for 3 days and two each received 25 mg/day for 2 days. A fifth adult female fed untreated cat food served as a control. After the final treatment each female was fitted with a radio transmitter (Verts 1963) and released at her respective capture site. These five skunks were located daily, during daylight hours, by using radio-telemetry, and were recaptured 3 to 4 weeks after the release dates and sacrificed to determine the effect of DES on developing embryos.

Effect of DES on Adrenal Weights

Two juvenile females that received 100 mg of DES each and two that received 400 mg of DES each were treated in captivity, and one juvenile female given no DES served as a control; all were fitted with radios and released on the Shannon Study Area in Carroll County. Each skunk was released at its point of capture. Each skunk was recaptured at 3 weeks posttreatment and sacrificed the day of recapture to collect and weigh its adrenals.

Activity Patterns of Radio-tagged Skunks

The effect of DES on the daily movement patterns of wild skunks was

studied during the summer of 1966. For this experiment, the five juvenile females used to test the effect of DES on adrenal weights, five juvenile males (four treated and one control), and two adult males (one treated and one control) were fed DES in cat food during a 3- to 4-day period in captivity. Treated skunks received a total dose of either 75 mg., 100 mg. or 400 mg. of DES. After treatment, each of the 12 skunks was fitted with a radio transmitter and released where captured, near Shannon, Illinois. Skunks were located by radiotelemetry and their positions plotted on maps once during daylight hours and one to three times during the night at least 5 days per week. An attempt was made to radio-track each skunk for 15 days following its release.

Field Tests With Wild Population

The field test to determine the effect of DES on a wild skunk population was conducted on a contiguous 46,000-acre area in southeastern Carroll County during 1965 and 1966. Tallow baits containing DES were prepared according to the procedures given by Ballantyne and O'Donoghue (1954) and Balser (1964). About 9,000 baits were distributed each year at about 650 stations. Baits were placed at stations once during March and once during April in both 1965 and 1966 (Table 1). Bait stations were established at ¼- to ½-mile intervals around each Section (640 acres). The baits were placed near culverts or fencerows along roadways. Seven, ½-ounce baits, each containing 85 mg DES, were placed at each station. All stations were examined for bait consumption and evidence of feeding within 10 days after the baits were distributed.

Skunks were collected on the DES-treated area, and on a control area located 5 miles south of the treated area, during May through August of 1965 and 1966. No. 1½ steel traps set in or near road culverts were used to capture skunks. Equal trapping effort was used for each area. Each adult female was classified into one of the following groups after gross examination of the mammae; (1) no sign of lactation, (2) signs of recent lactation, or (3) questionable lactation; the latter included skunks with long, pigmented teats but without milk. The ovaries and uteri of females and the testes of males, captured during 1965 and 1966, were removed, cleaned, and weighed the days the skunks were captured. The weights of the paired adrenals were obtained from the sample of skunks collected during 1966.

The March-April period was selected for placing baits in the field because the number of skunks is at a seasonal low, natural foods of skunks are in short supply, plant cover along roadsides in northern Illinois is sparse, and the movements of skunks in early spring are more extensive than in winter. The height of the breeding season of skunks in northern Illinois occurs during early March (Verts 1967:109). Thus a majority of the females were expected to be pregnant when the DES-baits were placed in the field.

Some disadvantages of placing baits in the field during early spring in northern Illinois include the possibility of low temperatures during March, which may result in more restricted movements of skunks than in summer, occasional snowfalls which may cover baits and result in reduced consumption, and poor road conditions.

Results

Reproduction of Adult Females

Four of the five adult radio-tagged females were recaptured alive 3 to 4 weeks after their release. One treated female resorbed her entire litter and two were resorbing at least one embryo each. The single control showed six viable fetuses. All four females appeared in excellent condition when recaptured. The fourth treated female died 29 days after the treatment. This female was barren, and was positive for rabies as determined from mouse inoculation and FA tests.

Adrenal Weights

Each of the five juvenile radio-tagged females (four treated and one control) was recaptured 3 weeks after treatment. The weight of the paired adrenals of the untreated female was 406.5 mg, whereas those of the four treated females ranged from 435.3 to 719.9 mg and averaged 590.1 mg.

Activity Patterns of Radio-Tagged Skunks

The 12 radio-tagged skunks used to test the effect of DES on the activity patterns of skunks were radio-tracked for at least 1 week each. There was no evidence that DES influenced their daily activity patterns. All radio fixes obtained during nocturnal hours for each treated and control animal were classified as *nighttime moving*. This means that the skunks were active at night and had moved from their respective daytime retreats. The tracking data showed considerable individual variation in the size of the activity areas used by the 12 radio-tagged skunks, but variation probably reflected the lack of sufficient tracking data rather than a real difference in size of activity areas among skunks. The data indicated that the movements of DES-treated skunks were not restricted after treatment and may have been more extensive than the movements of untreated skunks.

Field Test with Wild Population

More than 88 percent of the DES-baits distributed in 1965 and 1966 for the field test were taken within 10 days after placement (Table 1). Evidence of bait consumption by identified species was neglible during both years, and direct evidence of consumption of baits by skunks was not obtained. However, feeding trials conducted with three captive skunks showed that skunks readily ate the tallow DES-baits. Six tallow baits were placed near the den entrances of each of two radio-tagged skunks on one day during the late winter, 1964. The majority of the bait material was consumed at each den, and was believed to have been taken by the tagged skunks.

Thirty-three striped skunks were captured on the DES-treated area, and 20 were captured on the control area during the May-August period in 1965. In the following year, 24 and 26 skunks were captured on the treated and control areas, respectively. Based on the number of skunks captured per 1,000 trap-nights, there was not a marked change in the number on either the treated or the control area from 1965 to 1966 (Table 2).

Examination of adult females in the May-June sample indicated that a greater proportion of the females from the control area were lactating as compared with females from the treated area (Table 3), but the differences in the number of placental scars per female between skunks from the treated area and those from the control area were not significant ($P>0.05$). Likewise the differences in the size of the gonads of both males and females between samples of skunks from the two areas (1965 and 1966) and the differences in the weight of the adrenals for skunks from the treated and control areas (1966 only) were not significant ($P>0.05$).

DES had no apparent effect on the general behavior patterns of radio-tagged skunks. However, movement data were not obtained for DES-treated females during lactation. Verts (1967:60) reported that a lactating female skunk did not travel more than 360 yards from her den during the period May 25-June 2, but by June 12, when her offspring were older, she was often radio-located ½ mile from her den. This

TABLE 1.—Distribution, number, and percent of tallow DES-baits taken on a contiguous 46,000-acre (12 miles x 6 miles) in southeastern Carroll County, Illinois.

Year	Dates Baits were Distributed	Number of Baits	Number of Bait Stations*	Percent Baits Taken†
1965	March 11-16 April 9-24	8,904	636	88.7
1966	March 8-12 April 1- 9	9,324	666	93.0

* Seven baits were placed at each station (600 mg DES/station) during each of two distributions during each year.

† Stations were revisited within 10 days after each distribution.

TABLE 2.—Number of skunks captured on the DES-treated area and on a control area during May-August in 1965 and in 1966.

	Treated				Control			
Year	Adult	Juve-nile	Total	Skunks per 1,000 Trap-nights	Adult	Juve-nile	Total	Skunks per 1,000 Trap-nights
1965	30	3	33	25	18	2	20	15
1966	20	4	24	26	23	3	26	28

TABLE 3.—Reproductive characteristics of adult female skunks from a DES-treated and a control area during May-August, 1965 and 1966. Numbers in parentheses refer to the adult females captured during the May-June period.

Year and Area	Number of Adult Females Captured	Females Showing Definite Sign of Nursing		Number of Females with Viable Fetuses	Females with Placental Scars		Mean Number of Placental Scars/ Female	Mean Size of Ovaries (LxW)
		No.	Percent		No.	Percent		
1965								
Treated	17 (15)	10 (10)	58.8 (66.7)	1	14	82.4	6.6	45.6 mm
Control	8 (8)	6 (6)	75.0 (75.0)	0	8	100.0	8.3	47.5 mm
1966								
Treated	9 (5)	3 (2)	33.0 (40.0)	0	9	100.0	6.7	54.1 mm
Control	8 (3)	4 (2)	50.0 (66.7)	1	6	75.0	6.8	63.0 mm

suggests that the daily movement pattern of lactating females may differ significantly from that of females not tending young.

The data acquired from the field trials did not reveal a significant reduction in skunk productivity by the treatment used. More field tests, conducted under a variety of conditions and perhaps using more effective chemosterilants, are needed to determine whether the use of chemosterilants is a feasible approach to suppressing productivity in wild striped skunks.

Part of the explanation may lie in the fact that we had no way to determine which skunks consumed baits or how much DES a skunk had ingested. A laboratory study made concurrently with this field study gave inconclusive results because of small sample sizes, but captive females receiving single oral doses of DES ranging from 26.3 mg/kg to 367.7 mg/kg produced 0.5 living offspring per/living female for 8 treated females while six control females produced 1.7 living offspring per female.

Acknowledgments

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PHOSPHATES IN PEORIA LAKE A QUANTITATIVE AND QUALITATIVE EVALUATION OF A NUTRIENT IN NATURAL WATERS

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ABSTRACT. — Peoria Lake, a 13-mile long expansion of the Illinois River, lies approximately midway between Chicago and the Mississippi River on the Illinois Waterway. Its waters are substantially augmented by a variety of soluble and suspended materials from municipal and industrial wastes and from agricultural runoff both directly and from tributary streams.

A study of the chemical and biological characteristics of Peoria Lake was conducted from March to October 1967. Samples were collected on 25 dates from 9 stations at a standard depth of 3 feet. Laboratory analysis of soluble and particulate fractions of ortho-, poly-, and organic phosphate forms has permitted an evaluation of the various phosphates found in these natural waters.

The phosphorus budget of Peoria Lake for the period of study showed the mean daily phosphate load for all fractions and forms entering the lake to be 77.2 $\times$ 10^3 pounds; total phosphate leaving the lake was 63.4 $\times$ 10^3 pounds. The net phosphate apparently retained in Peoria Lake was calculated to be a mean of 13.8 $\times$ 10^3 pounds per day, or 17% of that entering the lake. Possible mechanisms affecting this retention and the distribution of the various fractions and forms are discussed.

This report presents the results of a portion of a study conducted over a period of 30 weeks on Peoria Lake, a region of the Illinois River (Figure 1). The overall investigation dealt with a variety of physical, chemical, and biological parameters. This presentation will be concerned with 1) methods for determining phosphorus in natural waters and 2) the observed changes in the phosphorus fractions, dissolved and particulate, as well as in the forms, ortho-, poly-, and organic phosphate. The dynamics of phosphorus and the various phosphates are described in the context of a shallow, unstratified, eutrophic river-lake.

Peoria Lake has assumed greater importance in recent years, as has every readily accessible expanse of fresh water. The public water supply for the city of Peoria, Illinois, with a population of 133,000 is nominally of groundwater origin but is augmented by some pumpage of surface water. This is taken from an intake located at the Peoria Narrows on the downstream end of the lake. Recreational use of Peoria Lake for on-water sports has increased greatly with water skiing exhibitions and sailing regattas becoming frequent events during the summer months.

The Illinois River and its tributaries upstream of Peoria Lake receive domestic and industrial wastes from 11 cities with populations above 10,000, including the Chicago-Cook County area with approximately 8 million inhabitants.

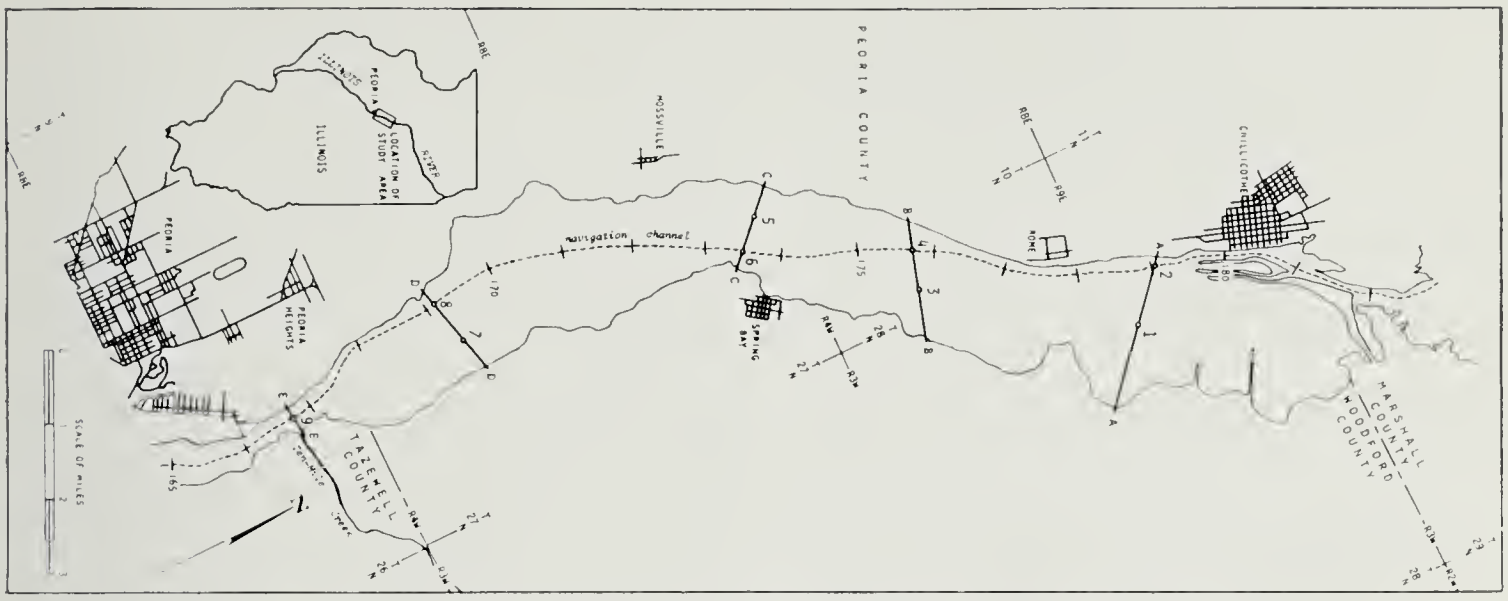

FIGURE 1. — Peoria Lake, transects and sampling stations.

On the basis of aquatic nutrients, the waters of Peoria Lake are as capable of supporting algal blooms today as in previous years when blooms occurred. Diatoms flourish, increasing turbidity and causing some coloration in the water. These organisms through photosynthesis also contribute dissolved oxygen.

Offensive organisms may still be detected by microscopic examination although their numbers are insufficient to impair water quality. A relationship between possible natural suppression of nuisance algae and concentrations of various phosphates is not known. A preliminary conclusion is suggested, in which the diminuation of certain algal populations is unlikely to be due to any paucity of phosphate.

The Illinois River and Peoria Lake

The Illinois River, of which Peoria Lake is a segment, is a stream of great age, flowing down an unusually flat gradient. As a subject for study the Illinois has long been favored, being referred to as ". . . the 'most studied' river in the world" (Mills *et al.*, 1966).

The Illinois Waterway extends across the state for a distance of 327.2 miles, of which 273 miles is the Illinois River. Beginning at the entrance of the Chicago River on Lake Michigan and flowing southward through the Chicago Sanitary and Ship Canal and a part of the Des Plaines River, the Waterway enters the Illinois River at its origin, the juncture of the Des Plaines and Kankakee Rivers.

Until 1938, four navigation dams divided the lower reach of the Illinois River into five pools, the last of which was at a common stage with the Mississippi River. At present six higher dams have increased the depth of the Illinois, aiding navigation and contributing to flood control. Peoria Lake is located in the third or Peoria Pool, 52.0 miles below Starved Rock Lock and Dam and 8.2 miles above Peoria Lock and Dam. Its present dimensions and other physical characteristics may be seen in Table 1.

Although it is formed by an expansion of the Illinois River, Peoria Lake (Figure 1) possesses features common to more orthodox lakes. The drainage basin of the Illinois River and its tributaries at Peoria Lake covers an area of 13,604 square miles. The lake bottom is covered with a thick layer of mud. A navigation channel 300 feet wide and from 9 to 21 feet deep runs the length of the lake. With a mean depth of less than 5 feet, Peoria Lake is unstratified throughout the year. Despite the general shallowness, very little emergent or floating rooted vegetation is to be found.

The whole pollutional load remaining after varying degrees of treatment that is borne by the upper reaches of the Illinois River is delivered to Peoria Lake. In recent years greater amounts of silt carried by the river have been attributed to erosional soil losses from

TABLE 1.—Physical Characteristics of Peoria Lake.

Length (Chillicothe to Peoria Narrows)	13.0 miles
Width	
Minimum	735 ft (0.139 miles)
Mean (area/length)	7,392 ft (1.400 miles)
Maximum	10,700 ft (2.027 miles)
Length-to-Width Ratio	9.3:1
Depth (at pool stage, 440.0 ft above mean sea level)	
Minimum	less than 1 foot
Mean (volume/area)	4.6 ft
Maximum (in navigation channel)	21 ft
Volume (at pool stage)	2.346×10^9 ft^3 (53.8×10^3 acre-feet)
During 3/13/67 - 10/3/67	
Minimum	2.3967×10^9 ft^3 (55.0×10^3 ac-ft)
Maximum	5.5401×10^9 ft^3 (127.4×10^3 ac-ft)
Area (at pool stage)	18.18 miles^2 (11,640 acres)
Retention time (estimated, 3/13/67 - 10/3/67)	
Minimum	1.60 days (38.4 hours)
Maximum	6.77 days (162.4 hours)

cultivated uplands. Increased barge traffic in recent years has also affected turbidity in the navigation channel and in adjacent shallower lake waters. Turbidity in certain areas of Peoria Lake is at times increased by phytoplankton, but suspended silt is the major constituent (Mills *et al.*, 1966).

Present water levels in Peoria Lake and throughout the Peoria Pool were established late in 1938 with the completion of the Peoria Lock and Dam. In the second year following stabilization of the pool at the present 440.0 feet above mean sea level, a marked increase in rooted aquatic plants appeared. Abundant growth continued for 10 years when it began to decline. Since the mid-1950's Peoria Lake has been virtually devoid of vascular aquatic plants. Turbidity has been suggested as a causative agent. However, in locations upstream where similar vegetative die-offs have occurred, decreased water turbidity has not produced a return of aquatic plants (Mills *et al.*, 1966). Whether this inhibition is in any way related to the relative absence of blue-green and other nuisance algae is not known. The increased silt load and absence of the phylogenetically higher plants have produced in Peoria Lake an extensive, shallow, turbid aquatic area unpunctuated by emergent vegetation.

LIMNOLOGICAL AND RIVER STUDIES

Background. Systematic chemical and biological examinations of Peoria Lake began with the work of Kofoid (1903). This led to his conclusion that the development of phytoplankton was limited by suspended silt that decreased light penetration of the water. He also believed that suspended decay bacteria propagated more in diminished light, consequently increasing the breakdown of suspended organic material.

Reoxygenation of the Illinois River after its passage through Peoria Lake was noted in a later study conducted by the Illinois State Water Survey (Greenfield, 1924). A further Survey investigation showed that decreased current velocity in Peoria Lake led to clarification of the water following sedimentation with an increase in algal growth in the clearer, slow flowing water (Boruff, 1930).

The U. S. Geological Survey found a decrease in suspended matter immediately below the Peoria Narrows and credited this to settling that had occurred in the lake (Collins, 1910).

Systematic sampling and analysis of water from the Illinois River at the water works intake near the Peoria Narrows was begun in October 1959 as part of the Water Quality Network of

the U. S. Public Health Service. This has continued to the present in the Water Surveillance System of the Federal Water Pollution Control Administration. Analytical results for the seven years from 1959-1966 showed total phosphorus to range from 0.3 to 0.8 milligrams per liter phosphorus (mg/1 P) with summer and winter means of 0.42 and 0.59 mg/1 P respectively. Dissolved phosphorus values ran from 0.2 to 0.6 mg/1 P with a summer mean of 0.30 and a winter mean of 0.48 mg/1 P. The U. S. Public Health Service considered the results of these analyses on the Illinois River at Peoria to yield phosphorus values among the highest found anywhere in the nationwide system (U. S. Public Health Service, 1963).

A recent paper on waste treatment and the Illinois Waterway System described a lowering of phosphate loadings between stations at Lacon and Peoria, a distance of 22 river miles of which 13 miles consisted of Peoria Lake (Hurwitz *et al.*, 1965). Values for total phosphate as phosphorus showed decreases from 15.54 tons to 10.30 tons per day, for ortho-phosphate from 12.22 to 7.04 tons/day P, and in the case of organic phosphate a slight increase from 2.00 to 2.06 tons/day P. These changes were suggested as being attributable to algal uptake followed by the sedimentation of dead organisms. Other means of natural phosphate removal mentioned were the immediate settling of insoluble phosphates and adsorption of phosphate on suspended matter followed by settling. In terms of concentration, the June-September 1960 average for total phosphate at the Peoria station was found to be 0.40 mg/1 P representing a decrease of 0.19 mg/1 P from the average for the same period at Lacon (Hurwitz *et al.*, 1965).

1967 Study. An examination of Peoria Lake was begun by the Water Quality Section of the Illinois State Water Survey in March 1967. Samples were collected at nine stations (Figure 1) at a uniform depth of about 3 feet.

Five transects were established: "A" immediately below Chillicothe at mile point 179 + 480 feet, with a shore to short width of 9195 feet; "B" below Rome, at mile point 175 + 5085 feet, width 7575 feet; "C" immediately below Spring Bay at the Spring Bay Narrows, at mile point 173 + 2880 feet, width 5785 feet; "D" above Peoria Heights, at mile point 169 + 390 feet, width 6630 feet; and "E" in Peoria Narrows, at mile point 166 + 3950 feet, width 735 feet.

At each of the first four transects two water samples were taken, one in the lake proper and the other from the navigation channel. Water was collected by Kemmerer sampler and placed in ½

Table 2.—Chemical Characteristics of Peoria Lake, March 13 - October 3, 1967.
(Constituents in mg/1)

	Mean	Range
Temperature	19.5°C	5.0 - 27.3
Turbidity (Jtu)	115	28 - 296
pH	8.19	7.57 - 8.69
Dissolved oxygen	5.6	1.4 - 15.3
Alkalinity (as $CaCO_3$)	165	136 - 213
Total hardness (as $CaCO_3$)	268	215 - 324
Iron (total)	3.21	0.69 - 13.01
Ferrous	0.58	0.16 - 1.89
Ferric	2.63	0.52 - 11.12
Fluoride (total)	1.08	0.17 - 2.06
Silica (total)	6.10	1.96 - 14.80
Nitrogen (total-N)	8.85	3.88 - 14.98
Nitrate (NO_3-N)	4.33	1.65 - 11.12
Ammonia (NO_3-N)	1.15	0 - 5.45
Organic-N	3.37	0.64 - 9.84
Phosphorus (total)	1.13	0.47 - 3.02
Orthophosphate-P	0.84	0.25 - 2.30
Polyphosphate-P	0.15	0 - 0.67
Organic Phosphate-P	0.14	0 - 0.58

gallon polyethylene bottles. Sampling runs were begun up-lake and finished at transect "E" near the laboratory, minimizing the time between collection and preparation of the samples for analysis.

Analyses using wet chemical methods were conducted for total and ferrous iron, nitrate, ammonia, organic nitrogen, total and reactive silica, fluoride, alkalinity, hardness, pH, and turbidity, as well as for phosphorus. Measurements of dissolved oxygen were made with a galvanic cell oxygen probe. Water clarity estimations by Secchi disk plus water and air temperature measurements were made in the field. Summary results of these parameters are presented in Table 2. Biological analyses consisted of total plankton counts with differential determinations of green algae, blue-green algae, flagellates, and diatoms.

Sample collection was begun in March and repeated on a weekly and occasionally bi-weekly basis until October. In all, 225 samples were collected on 25 dates over a 30-week period at 9 stations on Peoria Lake.

Hydrologic records of water flow rates and river stages were obtained from the U. S. Army Corps of Engineers and the U. S. Geological Survey.

Methods

Sample preparation. Raw water samples were delivered from Peoria Lake as quickly as possible, the maximum time being three hours and the minimum less than one hour. Well shaken, unfiltered samples were used for total phosphorus, total inorganic phosphate, and total orthophosphate analyses.

Samples for dissolved phosphorus, dissolved inorganic phosphate, and dissolved orthophosphate analyses were prepared by filtration through 0.45 μ membranes immediately upon receipt of the samples in the laboratory.

Phosphorus analysis. A review of current analytical methods for orthophosphate, total inorganic phosphate, and total phosphorus determinations was conducted before the Peoria Lake study was undertaken.

The customary procedure for phosphorus analysis involves the actual measurement of orthophosphate ($—PO_4^{-3}$). Any other forms of phosphate must be converted to orthophosphate for analysis.

Total phosphorus. In the analysis of total phosphorus in natural water it is necessary not only to hydrolyze all condensed phosphate but to oxidize organic phosphate. In surface waters, amounts of condensed and organic phosphates may be significant portions of the total phosphorus.

Probably the most widely tried method for total phosphorus is that presented in "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association, 1965), though doubts have been expressed about the adequacy of its digestion technique. If this method were restricted to groundwater or to surface waters lacking significant amounts of organic matter it might be adequate.

Although the perchloric acid technique of oxidation does release all polyphosphate and organic phosphate, the hazards of its use on many surface water samples containing varying amounts of organic matter have made it undesirable (Jenkins, 1967; Sanning, 1967).

Potassium persulfate was suggested as an oxidizing agent by several authors. After a series of comparative trials, the digestion technique detailed by Gales *et al.* (1966) was selected. This was used in conjunction with the acidic molybdate solution of Robertson (1960) and the potassium antimonyl tartrate solution

of Edwards *et al.* (1965). Ascorbic acid was used as the reducing agent for final color development (Menzel and Corwin, 1965).

Orthophosphate. Though several variations may be used, the determination of orthophosphate began with the addition of an acidic molybdate solution to an aliquot of sample. Free, dissolved PO_4 reacts with the molybdate giving 12-molybdophosphoric acid. A reducing agent was then added causing the formation of blue phosphomolybdic acid. The intensity of the resulting color is directly proportional to the amount of orthophosphate available for reaction (American Public Health Association, 1965). The color was measured photometrically at a wave length of 880 mμ in a Bausch and Lomb Spectronic 20 spectrophotometer-colorimeter.

The method selected for orthophosphate was that suggested by Edwards *et al.* (1965).

Aliquots of 10 ml diluted to 50 ml with double distilled water were used routinely on Peoria Lake water samples because of the high phosphorus concentration.

Total inorganic phosphate. In a number of different methods tested condensed polyphosphate were more or less completely hydrolyzed. Problems of accuracy and reproducibility made most of them unsuitable. The method of Robertson (1960) in which $Bi(NO_3)_3$ and nitric acid were used to hydrolyze polyphosphate provided the best procedure. Complete hydrolysis of known concentrations of various polyphosphates was found. The use of dry ascorbic acid as the reducing agent gave very good color development and consistent reproducibility of standard curves. A Lambert-Beer curve that lay in the same range as the previous orthophosphate curve was developed. Standards were run with each group of samples and recovery of known amounts of standard was found to be better than 99%.

All glassware including pipettes and reagent bottles were washed in a hot sulfuric acid-nitric acid mixture and rinsed only in distilled water. Polyethylene sample and storage bottles were washed in tap water then rinsed in dilute hydrochloric acid followed by rinsing in distilled water. No synthetic detergent washing compounds were used on any glassware employed in phosphorus analyses or in sample collections.

Total phosphorus, total inorganic phosphate, and orthophosphate were analyzed directly. The difference between total inorganic phosphate and orthophosphate was considered polyphosphate. Organic phosphate was regarded as the difference between total phosphorus and total inorganic phosphate. Total phosphorus and phosphate analyses were run on filtered and unfiltered samples. Dissolved fractions were the result of analyses on filtrate samples. Particulate fractions were the difference between total and dissolved values. Included in Table 3 is a summary of the procedures used to differentiate various fractions and forms of phosphorus in the course of the study.

Statistical evaluation. A two-way analysis of variance technique (ANOVA) was used to determine whether significant differences existed

TABLE 3.—Methods Used for Analyses of Phosphate-Phosphorus Fractions and Forms.

Fractions	Sample	Pretreatment	Color Reaction
TP Total phosphorus	unfiltered	Persulfate oxidation	Ascorbic acid reduction to molybdate blue
DP Total dissolved phosphorus	0.45 μ membrane filtered	Persulfate oxidation	Ascorbic acid reduction to molybdate blue
PP Total particulate phosphorus	by difference (Total phosphorus — total dissolved phosphorus)		
Forms			
TO Total ortho-phosphate-P	unfiltered	none	Ascorbic acid reduction to molybdate blue
DO Dissolved ortho-phosphate-P	0.45 μ membrane filtered	none	Ascorbic acid reduction to molybdate blue
PO Particulate ortho-phosphate-P	by difference (Total ortho-phosphate-P — dissolved ortho-phosphate-P)		
TPo Total poly-phosphate-P	unfiltered	$Bi(NO_3)_3$ hydrolysis	Ascorbic acid reduction to molybdate blue ——> total inorganic phosphate-P (TIP) TIP — total ortho-phosphate-P — TPo
DPo Dissolved poly-phosphate-P	0.45 μ membrane filtered	$Bi(NO_3)_3$ hydrolysis	Ascorbic acid reduction to molybdate blue ——> dissolved inorganic phosphate-P (DIP) DIP — dissolved ortho-phosphate-P — DPo
PPo Particulate poly-phosphate-P	by difference (Total poly-phosphate-P — dissolved poly-phosphate-P)		
TOr Total organic phosphate-P	by difference (Total phosphorous — total inorganic phosphate-P)		
DOr Dissolved organic phosphate-P	by difference (Total dissolved phosphorus — total inorganic phosphate-P)		
POr Particulate organic phosphate-P	by difference (Total organic phosphate-P — dissolved organic phosphate-P)		

between the mean phosphorus concentrations for different sampling stations and sampling dates. The test showed whether the lake was homogeneous without isolating differences between means. The Student's t-test was used to indicate the station or stations responsible for any differences.

Linear product moment correlations were computed to: 1) relate concentrations of one fraction or form to another, and 2) relate phosphorus concentrations to turbidity and plankton.

A relationship between two variables may exist, although an extraneous factor may affect this relationship more than one variable affects another. To give numerical expression to the relationship between two variables, correlation coefficients were used. A significant correlation is shown by the degree of approximation to 1.0. Such a correlation indicates similar behavior and from this, one variable may be predicted from another. With this in mind all correlations were checked for significance using a specialized Student's t-test. Confidence intervals were used for predicting the range of each correlation when deemed appropriate (Natvella, 1963).

All statistical tests were computed for the 95% level of confidence.

Phosphate-Phosphorus Concentrations

Comparisons based upon sampling stations. It was necessary to determine whether Peoria Lake was homogeneous through all nine sampling stations for each phosphate-phosphorus fraction and form before making comparisons between them. An analysis of variance showed only total and dissolved organic phosphate and dissolved polyphosphate to be homogeneous in concentrations throughout the lake.

The Student's t-test showed that concentrations of total and dissolved orthophosphate in the first six sampling stations were statistically similar at each two adjacent stations and also in the last three stations. This appeared to separate Peoria Lake into two segments: an upper segment including transects A, B, and C (stations 1-6), and a lower segment with transects D and E (stations 7-9).

Data for the entire 30-week study yielded mean values shown in Table 4 for each fraction and form at each sampling station. These means show the most abundant fraction to be dissolved phosphorus, comprising 64% of the total phosphorus. The major phosphate form was orthophosphate, which made up 75% of the total.

Changes in concentration with down-lake movement are depicted in Figure 2. As water moved from transect A to E there was a reduction in the phosphorus concentrations of most fractions and forms. This trend was gradual from transect A to C, with mean total phosphorus values decreasing from 1.26 to 1.17 mg/1 PO_4—P. There was an increased rate of change in concentration for most forms and fractions from transect C to D where the mean value for total phosphorus decreased from 1.17 to 0.93 mg/1. A virtually constant level was shown from transect D to E where a mean value of 0.94 mg/1 PO_4—P was found for total phosphorus.

When fractions and forms of total phosphorus were expressed in terms

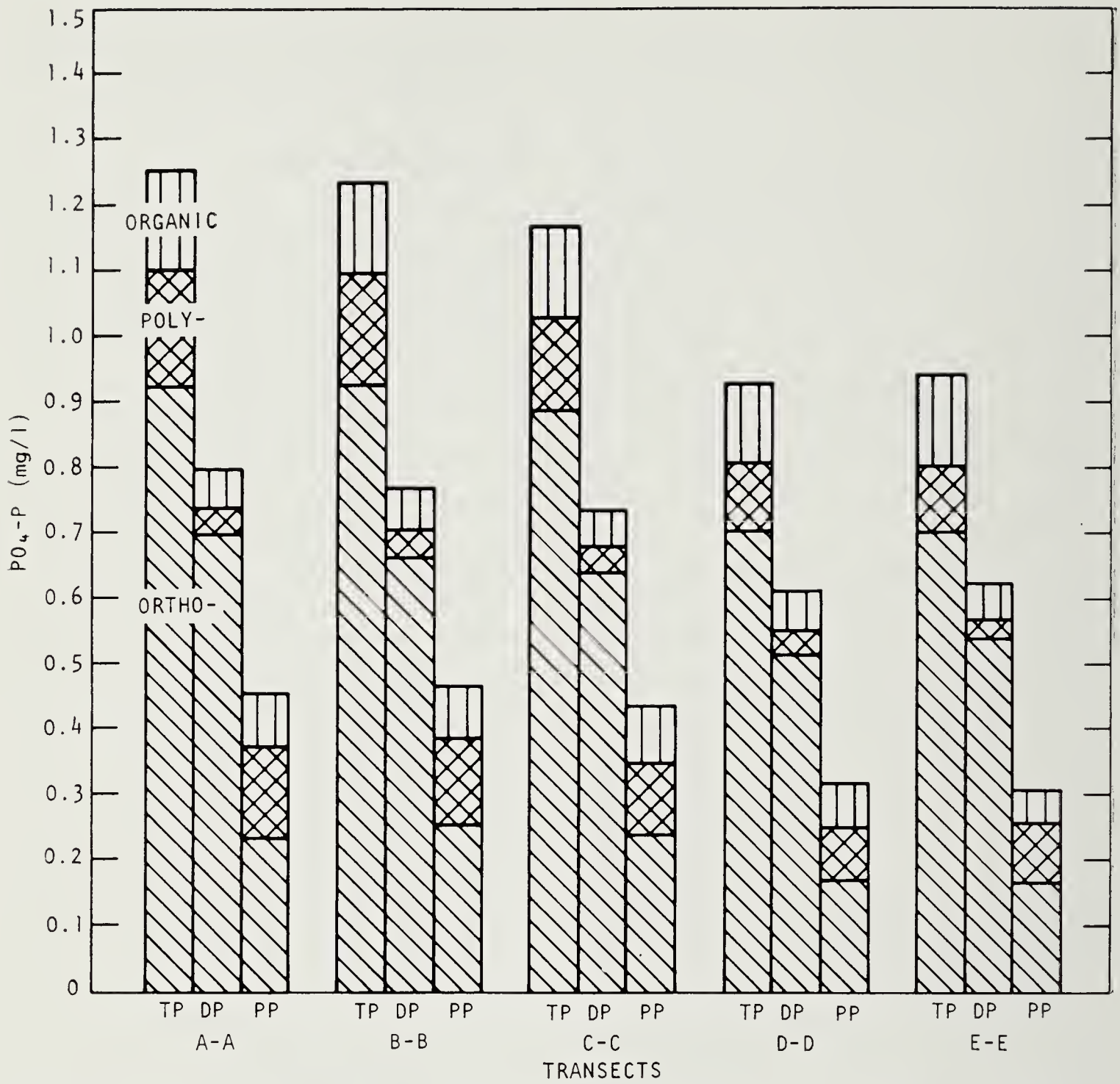

FIGURE 2. — Total, Dissolved, and Particulate Fraction Concentrations of Ortho-, Poly-, and Organic Phosphate at Each Transect.

of percent, a slight increase was found in dissolved phosphorus with a resultant reduction in the particulate phosphorus moving down-lake from transect A to E. This is shown in Table 5. It was also apparent that there was no appreciable change in the percentage of total ortho-, poly-, or organic phosphate forms from transects A through E. The distribution of forms on a mean percentage basis was observed to be 75, 13, and 12 percent for total orthophosphate, polyphosphate, and organic phosphate respectively.

As shown in Table 6, the percentage of dissolved and particulate orthophosphate remained nearly constant from transect A to E, with dissolved orthophosphate making up 74% of the total orthophosphate. An overall increase in dissolved polyphosphate and dissolved organic phosphate was found in moving down-lake. The mean for dissolved polyphosphate was 26% of the total

TABLE 4.—Means of All Forms and Fractions at Each Station on All Sampling Dates with Total Station Means and Standard Deviations (values in mg/1 P)

Station	Total P			Ortho-PO_4-P			Poly-PO_4-P			Organic-PO_4-P		
	Total	Diss.	Part.	Total	Diss.	Part.	Total	Diss.	Part.	Total	Diss.	Part.
1.....	1.19	.70	.49	.86	.60	.26	.19	.04	.15	.15	.05	.10
2.....	1.33	.89	.44	1.00	.78	.22	.18	.04	.14	.17	.07	.10
3.....	1.14	.68	.46	.83	.58	.25	.17	.03	.14	.14	.06	.08
4.....	1.34	.87	.47	1.03	.76	.27	.17	.04	.13	.14	.07	.07
5.....	1.20	.73	.47	.89	.65	.24	.15	.03	.12	.15	.04	.11
6.....	1.15	.74	.41	.89	.64	.25	.15	.04	.11	.12	.06	.06
7.....	.87	.56	.31	.63	.48	.15	.13	.03	.10	.12	.05	.07
8.....	.99	.66	.33	.77	.56	.21	.10	.04	.06	.13	.05	.08
9.....	.94	.63	.31	.70	.53	.17	.12	.04	.08	.13	.06	.07
Mean....	1.13	.72	.41	.84	.62	.22	.15	.04	.11	.14	.06	.08
Standard Deviations...	±.41	±.31	±.20	±.36	±.30	±.13	±.09	±.04	±.09	±.09	±.04	±.07

polyphosphate, and dissolved organic phosphate was 41% of the total organic phosphate. Thus, it appeared that the percentage increases in dissolved polyphosphate and dissolved organic phosphate were responsible for the previously mentioned percentage increase in dissolved phosphorus.

The percentage distribution of ortho-, poly-, and organic phosphate in dissolved and particulate fractions is shown in Table 7. From this it appears that there was little percentage change in the dissolved forms of ortho-, poly-, and organic phosphate down-lake. There were, however, some changes in percentage in particulate forms with a general increase in particulate orthophosphate and a decrease in particulate polyphosphate. Particulate organic phosphate percentages did not change appreciably.

It can be concluded that as water moved down-lake there was little change in the percent distribution of the three forms of total phosphorus, i.e., ortho-, poly-, and organic. This was not true of the particulate forms

TABLE 5.—Down-Lake Changes in Percentage of Total Phosphorus.

Transect	% Diss.	% Part.	% Ortho-	% Poly-	% Organic
A.....	63	37	74	14	12
B.....	62	38	75	14	11
C.....	63	37	76	12	12
D.....	66	34	75	12	13
E.....	67	33	74	12	14
Mean.....	64	36	75	13	12

TABLE 6.—Down-Lake Changes in Fraction Percentage of Phosphate Forms.

Transect	Ortho-PO_4		Poly-PO_4		Organic PO_4	
	% Diss.	% Part.	% Diss.	% Part.	% Diss.	% Part.
A	74	26	22	78	39	61
B	72	28	21	79	44	56
C	72	28	23	77	35	65
D	74	26	30	70	41	59
E	76	24	33	67	45	55
Mean	74	26	26	74	41	59

of ortho- and polyphosphate however. Changes did occur as were reflected by a possible exchange between the particulate and dissolved phosphates. This was especially noticeable in poly- and organic phosphate fractions.

Interrelationships. Correlation coefficients between two variables were derived in order to determine which fractions and forms behaved similarly. Correlations with the greatest similarity were found between 1) TP:TO:DP:DO, 2) TPo:PPo, and 3) TOr:POr. All significant correlations are shown in Table 8.

Seasonal comparisons. Peoria Lake displayed an apparent alternation between river and lake characteristics with seasonal changes in flow.

An analysis of variance showed that a significant difference existed in the concentration of all phosphorus fractions and forms for all 25 sampling dates in the 1967 study. On the basis of changes in flow rates, retention times, and water temperatures it was found convenient to divide the study into three periods (Table 9).

Figure 3 shows the mean phosphorus concentration of all stations at each sampling date during the study. The mean concentrations (Table 10 and Figure 3) of total, dissolved, and particulate phosphorus and total, dissolved, and particulate

TABLE 7.—Down-Lake Changes in Form Percentage of Phosphorus Fractions.

Transect	Dissolved			Particulate		
	% Ortho-	% Poly-	% Organic	% Ortho-	% Poly-	% Organic
A	87	5	8	50	30	20
B	87	5	8	54	29	17
C	88	5	7	55	25	20
D	86	6	8	53	25	22
E	84	6	10	53	25	22
Mean	87	5	8	53	27	20

TABLE 8.—Similarities Between Fractions and Forms Expressed as Correlation Coefficients

	Correlation Coefficient	95% Confidence Interval
DP:DO	0.99	0.98 - 1.00
TP:TO	0.98	0.96 - 0.99
TO:DO	0.97	0.93 - 0.99
TO:DP	0.97	0.93 - 0.99
TP:DO	0.96	0.90 - 0.99
TP:DP	0.96	0.90 - 0.99
TPo:PPo	0.95	0.88 - 0.98
TOr:POr	0.87	0.72 - 0.94
TP:PP	0.72	0.44 - 0.86
PP:POr	0.68	0.39 - 0.84
PP:PO	0.56	0.21 - 0.77
DP:PP	0.48	0.10 - 0.73
TOr:DOr	0.47	0.08 - 0.72
DP:PO	0.34	0.04 - 0.64

orthophosphate increased notably from the first period to the second, and from the second period to the third. There was little change in means for total, dissolved, and particulate polyphosphate and organic phosphate, though on a date-to-date basis an analysis of variance showed significant changes. Within total phosphorus and total orthophosphate the particulate fraction remained more nearly constant than did the dissolved fraction (Figure 3). Maximum levels of total phosphorus and total orthophosphate were produced primarily by increases in dissolved orthophosphate. Within the total polyphosphate form, the dissolved fraction remained more nearly constant and changes appeared to be due mainly to variations in the particulate fraction. Within the total organic form, both the dissolved and particulate fractions increased and decreased

TABLE 9.—Physical Characteristics of Different Seasonal Periods.

Periods 1967	Flow (10^3cfs)	Retention Time (days)	Water Temperature (°C)
(1) March 13 to May 29	26.2 (20.5 — 34.0)	1.86 (1.66 — 1.94)	11.6 (5.0 — 16.7)
(2) May 29 to August 14	10.9 (4.9 — 19.9)	3.21 (1.60 — 6.02)	24.1 (16.6 — 27.7)
(3) August 14 through October 3	5.4 (4.1 — 7.2)	5.55 (4.26 — 6.77)	20.6 (17.2 — 25.5)
Means for all periods	13.6	3.49	19.6

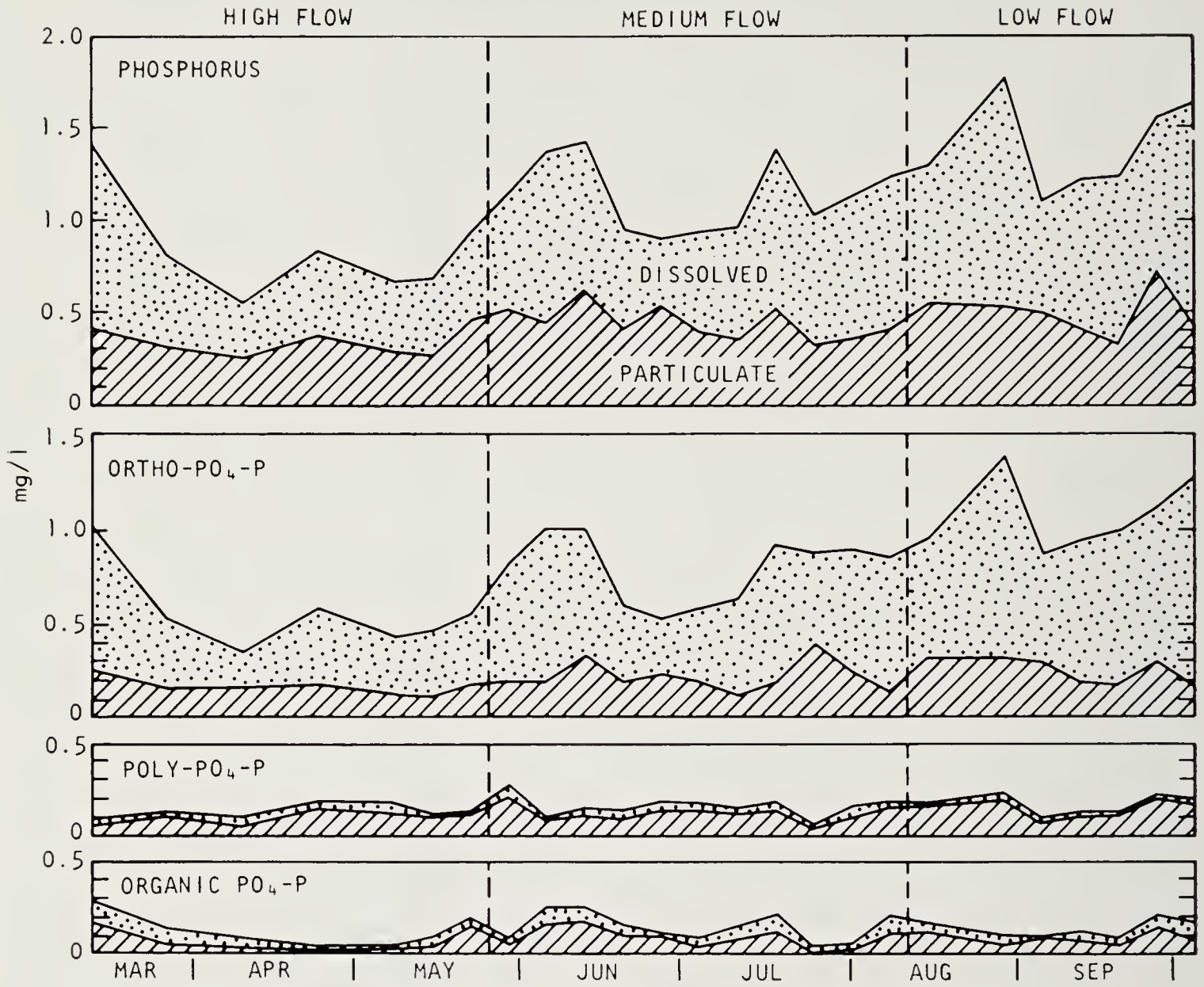

FIGURE 3. — Phosphate-Phosphorus Concentrations During Different Periods of Flow.

together. Therefore, major changes in total phosphorus were due to changes in dissolved orthophosphate, while the total poly- and organic phosphate fractions appeared somewhat interdependent.

From correlations of flow with phosphorus concentrations, it was found that only total and dissolved phosphorus and total and dissolved orthophosphate could be correlated with flow (Figure 4). The rate of concentration change was substantial from a flow of about 4,100 cfs to 10,000 cfs. During flows in excess of 10,000 cfs the rate of change was essentially constant. Flow appeared to have the greatest influence on dissolved orthophosphate as was thus reflected in the flow to total phosphate relationship.

Phosphorus, turbidity, and plankton. Concentrations of particulate phosphorus and particulate orthophosphate compared with turbidity showed moderately significant correlations. The correlation coefficient of mean particulate phosphorus to mean turbidity was 0.65 and mean particulate orthophosphate to mean turbidity was 0.64. The levels of phosphorus and turbidity were less in the lower lake segment than in the upper segment. These correlations suggest that phosphate, especially in the orthophosphate form, may be adsorbed on silt (Olsen, 1964; Muller and Tietz,

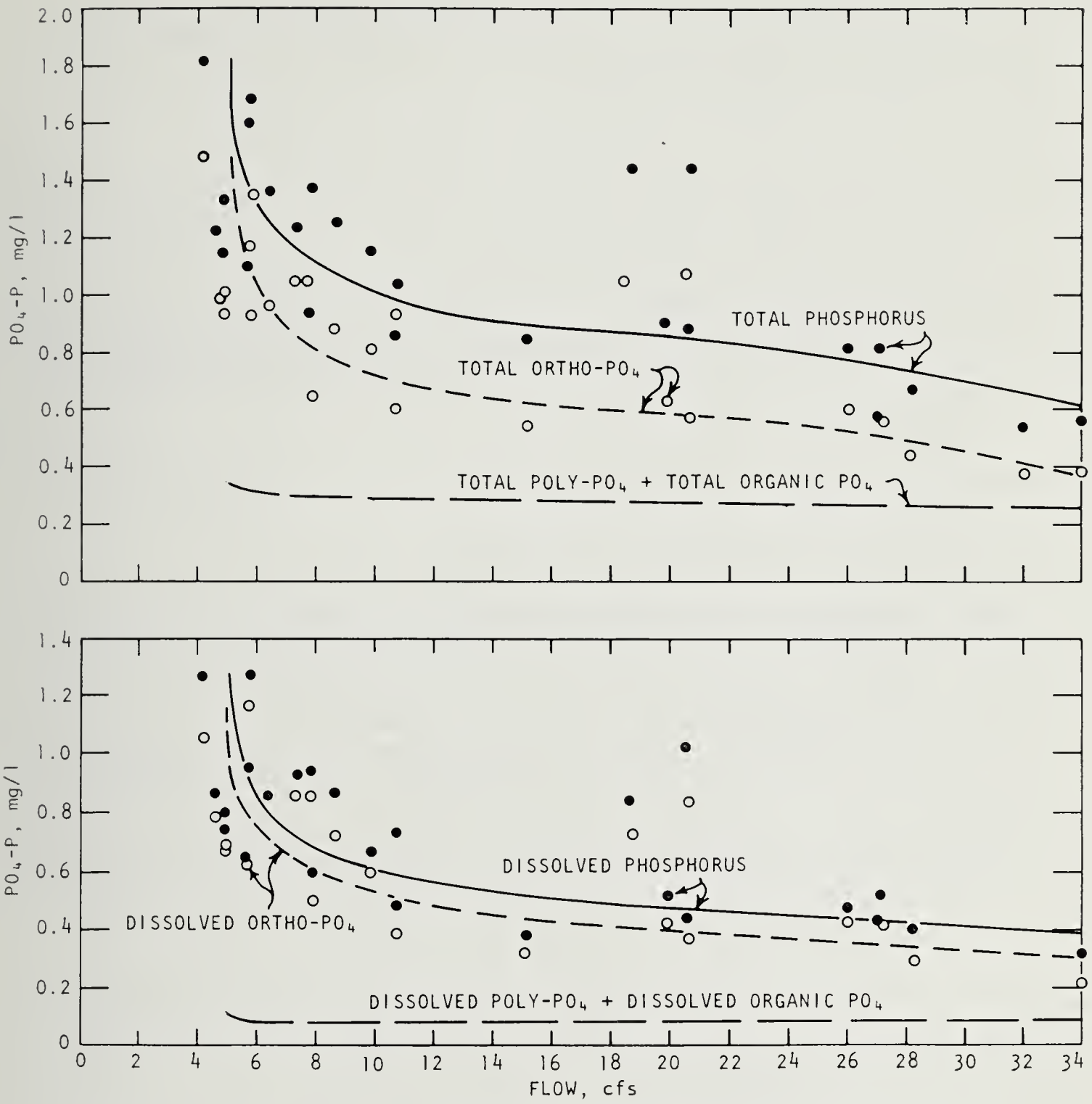

FIGURE 4. — Total and Dissolved Phosphate-Phosphorus Concentrations Expressed as Functions of Flow.

1966) and thus removed from the lake water by sedimentation.

Correlations of lower significance were found between mean total phosphorus and mean total plankton (correlation coefficient of 0.57) and between mean particulate phosphorus and mean total plankton (correlation coefficient of 0.40). It may be inferred that plankton had less of an influence on the phosphorus concentration than did some other factor. Plankton population increased two to three fold during the last 10 weeks of the study exceeding the level found in the first 20 weeks, while total phosphorus concentration increased only about 40%.

Phosphate loads. Flow rates, lake volumes, and phosphorus concentrations were used to calculate phosphate loads in terms of pounds per day. As Figure 5 shows, there was a decrease in phosphorus loadings in

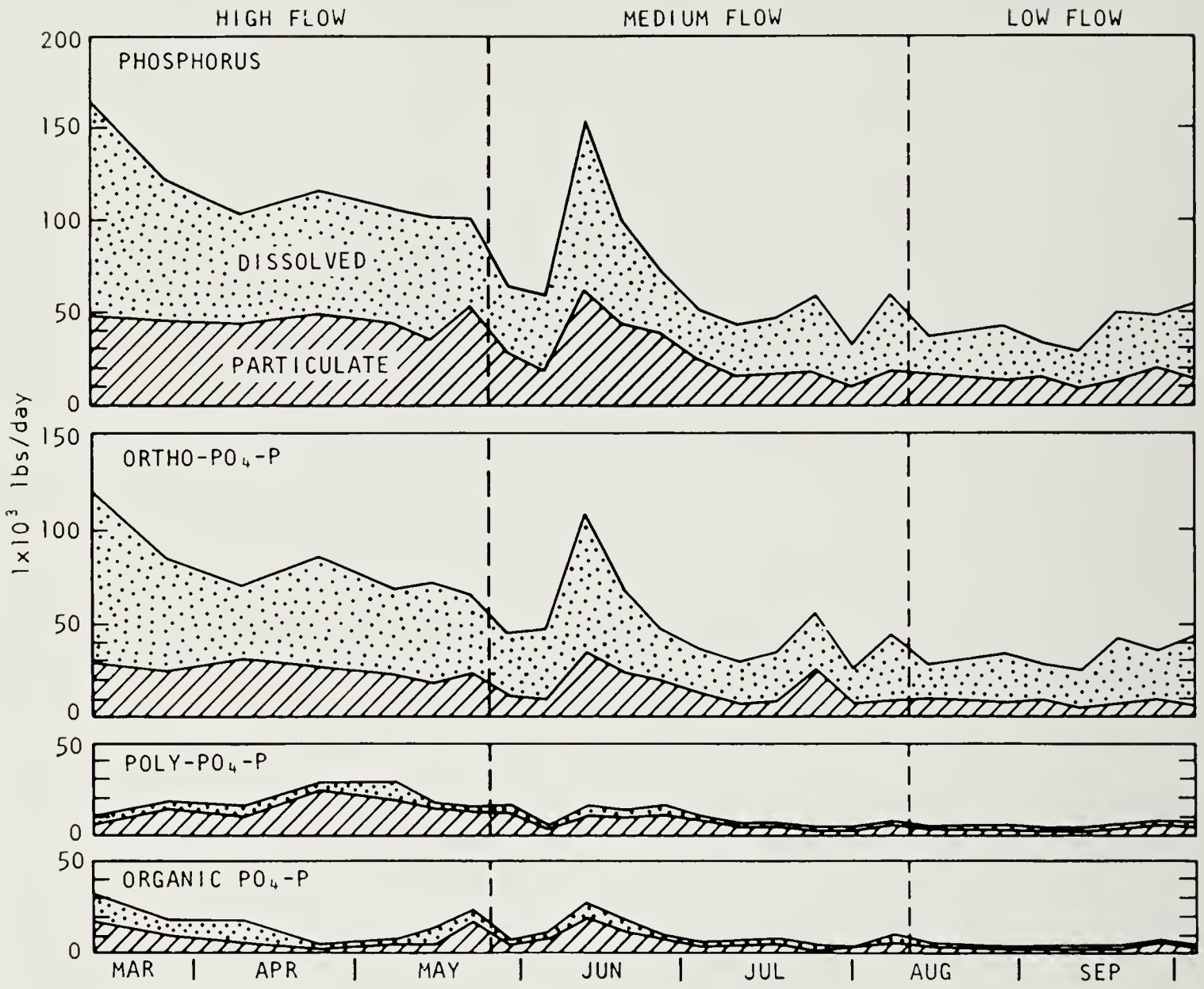

FIGURE 5. — Phosphate-Phosphorus Loads During Different Periods of Flow.

all fractions and forms from the first period to the second and from the second period to the third. This is accounted for by the fact that flow rate decreased much faster than phosphorus concentration increased. High flow rates are due principally to increases in surface runoff and to regulation by dams along the Illinois River.

Phosphorus budget. In calculating the phosphorus budget, mean phosphorus loads of the lower lake segment (stations 7-9) were subtracted from mean loads in the upper segment (stations 1-6). Since flow rate and retention time play a significant part in the phosphorus budget, these calculations were made for the third period of the study (from August to October 1967) when flow rate was lowest, retention time was highest, and Peoria Lake most resembled a true lake. Mean phosphorus loads retained and retention percentages were calculated for each phosphate form and fraction (Table 11). A comparison was made with means for the entire period of study.

During the third period the greatest retention, regardless of fraction, occurred in the orthophosphate form. Considerably less retention was found in polyphosphate, and even less in organic phosphate. This pattern was also seen in most of the mean values for the entire period of study.

TABLE 10.—PO_4-P Concentrations (mg/1) at Differing Periods.

	Period 1	Period 2	Period 3	Mean
Total P	.84	1.13	1.42	1.13
Total Ortho-PO_4-P	.58	.82	1.13	.84
Total Poly-PO_4-P	.13	.16	.16	.15
Total Organic PO_4-P	.13	.15	.13	.14
Dissolved P	.52	.69	.95	.72
Dissolved Ortho-PO_4-P	.42	.59	.88	.62
Dissolved Poly-PO_4-P	.03	.04	.03	.04
Dissolved Organic PO_4-P	.07	.06	.04	.06
Particulate P	.33	.43	.48	.41
Particulate Ortho-PO_4-P	.17	.23	.26	.22
Particulate Poly-PO_4-P	.10	.11	.13	.11
Particulate Organic PO_4-P	.06	.09	.09	.08

With few exceptions, a larger percentage of the phosphorus load was retained during the third period than was retained for the mean of all dates during the entire study. During the third period the form showing the highest percent retention was dissolved polyphosphate, though during the entire study particulate polyphosphate showed the highest percent retention. The means for the total 30 weeks showed a slight percentage gain for dissolved polyphosphate in water leaving Peoria Lake.

The mean daily load of total phosphorus entering Peoria Lake for the period of this study was 77,200 pounds, while that leaving the lake

TABLE 11.—Comparative Phosphorus Budgets for the Low Flow Period 3 and the Entire 30-Week Period of Study.

	Load retained (10^3 lbs/day)		Percent retained	
	Period 3	30-Week Mean	Period 3	30-Week Mean
Total P	11.4	13.8	25.9	17.9
Total Ortho-PO_4-P	9.1	9.5	25.4	17.3
Total Poly-PO_4-P	1.8	2.7	38.9	25.6
Total Organic PO_4-P	0.5	1.6	12.8	12.6
Dissolved P	6.6	5.8	22.2	12.7
Dissolved Ortho-PO_4-P	6.2	5.5	22.5	14.1
Dissolved Poly-PO_4-P	0.4	—0.1	40.0	—4.2
Dissolved Organic PO_4-P	0	0.4	0	8.9
Particulate P	4.8	8.0	31.8	25.7
Particulate Ortho-PO_4-P	2.9	4.0	34.2	24.2
Particulate Poly-PO_4-P	1.4	2.8	34.2	31.8
Particulate Organic PO_4-P	0.5	1.2	18.5	20.3

TABLE 12.—Comparative Date From Three Sources on Phosphate in Peoria Lake. (All values as mg/1 P)

	FWPCA 1959 - 1966	Hurwitz *et al.* June - Sept. 1960	1967 Study
Number of samples	350	25	225
Total phosphorus			
Mean		0.39	1.13 ± 0.41
Summer mean	0.42		
Winter mean	0.59		
Range	0.3 — 0.8		0.47 — 3.02
Dissolved phosphorus			
Mean			0.72 ± 0.31
Summer mean	0.30		
Winter mean	0.48		
Range	0.2 — 0.6		0.20 — 2.00
Total Orthophosphate mean		0.26	0.84 ± 0.36
Total Polyphosphate mean		0.05	0.15 ± 0.09
Total Organic Phosphate mean		0.08	0.14 ± 0.09

was 63,400 pounds. The net amount retained was 13,800 pounds per day or 17.9% of that entering the lake.

Comparison of 1967 data and previous data. Two major difficulties were found in placing current findings on phosphorus from the 1967 Peoria Lake study in historical perspective. First, only recently have phosphorus analyses been run on water samples from Peoria Lake. Previous sampling had generally been done only at the narrow outlet and in the area of station 9. Second, methods of filtration and analysis, especially for total inorganic phosphate and total phosphorus, have undergone many recent changes leading to higher total phosphorus values with a redefining of the phosphorus portion previously known as organic.

In a paper on phosphates in the Illinois Waterway System, Hurwitz *et al.* (1965) gave values shown in Table 12, here converted to phosphate-phosphorus. In addition, mean phosphorus concentrations for the period 1959-1966 based upon data published by the Water Pollution Surveillance System (U. S. Public Health Service, 1963) and the Federal Water Pollution Control Administration are presented for comparison with data from the 1967 Peoria Lake study.

Any lack of close comparability is suggested as being due as much to differences in methods of sampling, filtering, and analysis as to any real 2- to 4-fold increase in each phosphorus fraction and form in the lake.

Comparison with data from other bodies of fresh water. As the analytical data readily show, Peoria Lake is notably high in phosphorus content. A comparison of published concentrations of total phosphorus in a variety of lakes, parts of lakes, and reservoirs (Table 13) show Peoria

TABLE 13.—Comparative Levels of Total Phosphate in Fifteen Lakes and Reservoirs.

Lake	Year	Concentration, Means and/or Range (mg/1 P)	Reference
L. Santa Fe, Florida	1955	0.0016	Lackey & Putnam 1965
Fern L., Washington	1963	0.0043	Olsen et al. 1967
L. Dojran, Yugoslavia	1965	0.012	Petrovic 1965
L. Zoar, Connecticut	1955	(0.012—0.041)	Curry & Wilson 1955
L. Michigan, south basin	1954	0.013	Beeton 1960
L. Superior	1960	0.014	Beeton 1960
L. Huron, Saginaw Bay	1956	0.016	Beeton 1960
Slapy Reservoir, Czech	1959-60	(0.03—0.07)	Hrbacek 1966
Upper Klamath L., Oregon	1965-66	(0.03—2.0)	Miller & Tash 1967
Bantam L., Connecticut	1966	0.037	Frink 1967
L. Sebasticook, Maine	1965	(0.04—0.07)	Mackenthun et al. 1968
L. Erie, west end	1958	0.044	Beeton 1960
Sturgeon L., Ontario	1953-55	0.044	Neil 1958
Green L., Washington	1959	0.068	Sylvester & Anderson 1964
Peoria L., Illinois	1967	1.13 (0.47—3.02)	

Lake to have from 17 to 94 times the phosphorus concentration found in the other bodies of water. The first two examples cited, Lake Santa Fe, Florida, and Fern Lake, Washington, are oligotrophic and are included for comparison since their concentrations of total phosphorus are proportionally about as much below the other twelve lakes as Peoria Lake is above them.

Typical surface waters in Illinois in 1957 averaged 0.26 mg/1 P as total phosphate-phosphorus (Dietz and Harmeson, 1958). The Kaskaskia River, Illinois in 1960 showed concentrations to range from 0.08 to 0.30 mg/1 P (Englebrecht and Morgan, 1959). A recently published evaluation of the Ohio River gave as a 1966 maximum 0.39 mg/1 P (Ohio River Valley Sanitation Commission, 1967). All of these concentration levels were exceeded by the minimum total phosphorus concentration for Peoria Lake (Table 13).

With the exception of waters immediately adjacent to the outfalls of waste treatment facilities, Peoria Lake in 1967 showed some of the highest concentrations of total phosphorus yet reported.

This is also emphasized by a comparison of amounts of influent phosphorus from the drainage areas of three different lakes with that of Peoria Lake in 1967 (Table 14).

TABLE 14.—Phosphorus Input to Four Lakes.
(Mean pounds/square mile of drainage area/year)

Lake	lb P
Lake Sebasticook, Maine	63.5
Lake Mendota, Wisconsin	188
Upper Klamath Lake, Oregon	263
Peoria Lake, Illinois	2,050

SUMMARY

1. Laboratory methods and techniques used permitted expressing

phosphorus concentration in natural water in terms of its various forms — ortho-, poly-, and organic — as well as in the fractions — dissolved and particulate.

2. The mean total phosphorus concentration observed in Peoria Lake was 1.13 mg/1 P with a range of 0.47 — 3.02 mg/1.
3. Orthophosphate represented 75% of the total phosphorus, and 64% of the total phosphorus was in a dissolved state.
4. Differences in total phosphorus concentration with down-lake water movement and in weekly fluctuations were principally reflected by changes in orthophosphate concentration.
5. Degrees of interchange were observed in the percentage distribution of dissolved and particulate fractions during down-lake water movement. The changes in total dissolved and total particulate phosphorus were reflected by corresponding changes in dissolved and particulate poly- and organic phosphate.
6. The only notable down-lake change in the distribution of the various forms of phosphate, i.e., ortho-, poly-, and organic, was in particulate ortho- and polyphosphate.
7. Dissolved orthophosphate was the only fraction of any form that could be shown to be related to flow or seasonal changes.
8. Despite a very low retention time, total phosphorus removal within the lake averaged 17.9%, or about 13,800 pounds per day.
9. Although observed nutrient conditions in Peoria Lake appeared sufficient to support plankton populations, such did not occur.

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NOTES

PUBLIC AREAS: MULTIPLE USE OR MULTIPLE ABUSE?

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Virtually everywhere one looks the pressures for multiple use have resulted in violation of the original objectives established for areas set aside for public interests. We seem to have quickly forgotten or overlooked the past and present problems that resulted from only limited private interests such as mineral extraction, grazing, water supply, and timber harvest. Are we to anticipate that the current emphasis on public recreation on government-owned facilities will permit legitimate administration when employees are under pressure to think first of public relations and second of responsibilities to their profession and natural resources with which they are charged?

These comments are not to be interpreted as opposition to the necessity of providing recreational outlets for the public. On the contrary, full support is extended to such a program, but it is strongly suggested that it must be geared to well-organized, long-term planning; it cannot be a reflection of policy-making that results from emergency or panic. Unquestionably, present government holdings can contribute to these long-range plans; but we must recognize that only purposeful understanding and additional acreages can alleviate public abuse of limited holdings which reflect something of our heritage.

Although irresponsibility is readily inherent in previous resource management policies on national holdings, particularly in the West, the potential disaster to the concept that resources are to be managed for the greatest good of mankind for the longest period of time should be frightening to any responsible resource manager. Very suddenly the concept of multiple use has become prostituted by the public, the politicians, government administrators, and even professional foresters and biologists. The wildlife ecologist, soil conservationist, forest ranger, park naturalist, and many administrators talk more and more about public relations and less and less about conservation and management practices for resources with which each is charged. Needless to say, multiple use can be justified only when the original objectives of resource management are *always* given priority. Have we forgotten that the nature of resources rarely, if ever, permits compromise? Have we not learned that abstention or even moderation is a doctrine more justifiably administered than salvage programs? Have we not learned from past experiences that panacean programs created in an atmosphere of urgency are potentially catastrophic?

It has taken many years to show only limited returns from the efforts of various professional programs of resource management; forestry practices, however, are probably more advanced than many others. Seemingly, we have overlooked the status of these professions and the condition of resource management programs at the turn of this century. Now, within a period of one or two years we are on the verge of losing much that has been gained in the past 50 years. But, even more critical, the potential status of resource professions will be inevitably worse than ever previously experienced, if we tolerate the continuous brainwashing tactics of promoters of recreation. Instead of exercising professional leadership we are accepting assignments from the nonprofessional and ill-advised. Everything is rapidly being geared to tourism and a

fast buck, under the disguise of eloquent lip-service orations on public needs for recreational outlets. In fact, each of us, if an honest analysis of current situations is made, must recognize that we have been sold out by some of our prominent and trusted leaders. Surely, another "Silent Spring" is not to be required in order to bring some rectification to problems which responsible resource managers already know exist. Perhaps we need to re-educate ourselves, and surely the general public and state and national administrators, on the basic and original objectives of refuges, parks, wilderness areas, state and national forests, and scenic areas.

Justification for the restriction of general recreational uses of any public holdings should soft-pedal the fact that they were purchased from earmarked funds. Too frequently, negative reactions are stimulated when specialty interests are emphasized. There can be adequate justification made by appropriate and well-planned emphases on the real purpose for which a given area was set aside. The overall effects of hordes of people using areas which were established for biological or even scenic purposes must be demonstrated with facts, even if one has to emphasize poisonous snakes, or suggest that the original planned public use must be curtailed in the interest of maintaining the integrity of the primary purpose of an area. This suggests too that it may occasionally be necessary to admit that errors of judgment have been made in original ideas of land use. We must not attempt to hide behind professionalism if planning has not been well done.

If and when animal population controls become necessary on areas traditionally closed for harvest, every possible method outside of public hunting must be demonstrated. Should the latter become necessary, it must be clearly established that this does not represent an attempt to increase the amount of area for public hunting, but rather removal of animals in the interest of proper management of the area as a forest, refuge, or park. When removal of surpluses becomes necessary, regular or annual seasons should not become a practice, but wholesale removal at one time must be the objective so that public harvest is something that occurs at infrequent and even irregular intervals to avoid the establishment of a precedent which will result in uncontrollable public pressure.

Public relations cannot be tolerated as the basis for human destruction of the scientific and resource management purpose of an area. As biologists, technicians, and managers, we cannot afford the consequences of becoming servants of public interests which are not informed or educated as to the specific merits of restricted holding. If indeed multiple use is an attainable objective, then we cannot lose sight of what it really means. Too little research data are actually available to validate much of what already appears in multiple use programs. When we prepare for multiple use, there should be some idea as to the results that might be expected. Are we measuring the conditions of before and after management with organization so that clear-cut understandings can be derived? Or, are we entering programs of multiple use without knowledgeable appreciation of the potential good or bad that can be expected? Are we operating in an atmosphere of inquiry and freedom, or is it one of pressure and crisis? Are we really applying our skills as they are best known, or is it in ignorance, either by design or in honesty? Has the ground work really been established so that reasonable results can be anticipated? Who determines policy when it comes to management? Do you as technicians exercise any influence in final decisions? What are you willing to accept and still maintain professionalism? What research do you have underway, what have you done, or what results do you have available that guide the course of acceptable multiple use? I contend that such information may be far more useful at this stage than most anything that deals with forest or wildlife management. Do you r e a l l y understand recreation? Surely, the public does not, and my experience with professional recreationalists suggests that they do not understand people let alone anything about the resources they propose to exploit. I suggest that most of what is called outdoor recreation is nothing more than indoor activity moved outdoors.

Needless to say, I am fearful of the results of multiple use. The pressure of tourism and a fast buck are widely apparent. Unfortunately, much evidence suggests that this is being readily, and seemingly willingly, enhanced by so-called multiple use programs being established by state and national agencies of which each of us is a part. I am appalled at the misconception that is ap-

parent in the kinds of uses being made for recreation in what are truly scientific wonders. I'll mention no specifics but will demonstrate by saying that I find it hard to appreciate a gravel path and russian olive in a unique hilltop prairie. Forest areas are unique and especially important as frequently, if not regularly, they represent the last remnants of reasonably natural conditions. The Shawnee National Forests reflect many of these in such areas as Reed's Canyon, Little Grand Canyon, Pine Hills, Belle Smith Springs, Pomona Natural Bridge, Fountain Bluff, Drapers Bluff and Jackson Hollow but to mention just a few.

The time has come, in fact it may well have passed, when all professions dealing with natural resources should reevaluate the ethics reflecting their responsibilities as employees charged with safeguarding natural resources. Charged responsibilities do not allow for "lip-service" or bending to public whims. Who is supposed to know better or more about the needs of resource planning and management than we? If we cannot accept the charge of our professions, then it is high time that we do something about it. Like the manager of a baseball team, we must make the decisions, we must stay in command of the "public players"; we cannot forfeit our responsibilities without loss of our profession. Good public relations are important but this is not to be interpreted as doing what the public wishes. If so, it is an acknowledgment that they know better than we what our jobs are.

I take issue with the big push by many resource management agencies to provide recreation on every public holding; recreation is being over-emphasized at the expense of proper resource management. This comes at a time when we as researchers and managers are just coming into our own. It must be admitted that in many ways we are just now on the threshold of an understanding of some resource management problems, and for others we have not yet "arrived."

Many resource managers would do well to think more of management of the resources and less about whether the public has a place to harvest timber, farm, swim, camp, picnic, or hunt. In many areas we have not solved problems of public use but are already extending the opportunities for these problems, knowing full well the potential results.

It is high time that all professional resource agencies appraise the situation at hand. Let us examine the current status of our professional ethics. Are we moving forward at a pace representative of our current status of research, available monies, size of membership on a percentage basis of eligibility, and charged responsibility in the best, long-term interest of all mankind? Honesty may suggest that it will be difficult to establish an affirmative answer to these questions.

If we accept the philosophy that there is great need for the provision of recreational facilities for the public, then we as professionals should be planning in every way possible to provide these without endangering our first and foremost responsibility: namely, uninhibited, wise resource management. The first step is to establish unquestionably that many new areas must be obtained and designed specifically for this function. Secondly, we must *convince* our superiors and the public that certain types of areas now available, or to be obtained in the future, have little or nothing to offer to relieve the public recreational interests. Thirdly, we cannot permit recreation potential to become the basis for all future land acquisition. The needs for recreation and resource management must each be evaluated on the basis of individual merit; one must not be administered at the expense of the other. We cannot afford to condone or "buy" crash programs such as are being foisted upon us. As resource personnel we must be leaders and not followers.

Manuscript received July 18, 1968.

OBSERVATIONS OF *TAMIAS STRIATUS* FEEDING UPON *CONDYLURA CRISTATA*

JOHN N. KRULL
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ABSTRACT. — An unusual feeding behavior pattern on the part of eastern chipmunks is reported from upstate New York.

The eastern chipmunk, *Tamias striatus*, probably utilizes a diversity of food that is surpassed by few other mammals. These include a long list of plants from lichens to various parts of the higher spermatophytes, and a variety of both invertebrate and vertebrate animals. Included in the latter are such infrequent items as salamanders, snakes, and small, unwary birds. Occasionally these beasts even turn to cannibalism.

As far as is known, however, the literature on food habits of eastern chipmunks does not contain an account of these opportunists feeding upon either living or dead star-nosed moles, *Condylura cristata*. The purpose of this note, then, is simply to document a series of observations relating to this previously unreported feeding behavior.

On three different occasions during July of 1966 near Albany, New York, a freshly killed mole was placed in an area regularly frequented by several chipmunks. On each occasion a minimum of three different chipmunks completely devoured the softer parts of the mole within approximately one hour's time.

Reasons for this seemingly unusual feeding activity are not easy to enumerate. Simply the opportunist nature of the species may be more nearly the real explanation for this behavior than any other speculation. It might also be postulated that these chipmunks were attempting to supplement their diet in some way. A look at the surrounding area, however, revealed that a wide variety of foods were present. And the food items provided daily in a man-made feeding station also supplemented their natural diet.

Although these observations do not demonstrate that eastern chipmunks prey upon star-nosed moles, they do show that chipmunks will eat dead moles and that another item can be added to the list of food consumed by these animals. Since both chipmunks and moles occur throughout much of Illinois and other midwestern states, documentation of this rodent actually preying upon this insectivor will complete a most unusual feeding behavior pattern.

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HYDROGEN BONDING OF ALCOHOLS TO SUBSTITUTED PYRIDINES

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ABSTRACT.—The shifts of O-H stretching bands have been determined for alcohols hydrogen bonded to a series of substituted pyridines. A linear relationship exists between the shift of O-H wavenumber and the pK_a values of the bases. The shifts also correlate with previously reported O-D shifts for CH_3OD and other data for complexes of the bases.

Many correlations have been attempted between the wavenumber shift of the O-H stretching bands of alcohols hydrogen bonded to bases and other parameters associated with the hydrogen bonds (Pimentel and McClellan, 1960). Tamres, *et al.*, (1954) have shown that there is a linear relationship between the wavenumber shift of the O-D band of CH_3OD when hydrogen bonded to pyridines and the pK_a values of those bases. This was also shown for CH_3OH when some other nitrogen heterocycles were used (House, 1967). In this study, a variety of substituted pyridines were used with three alcohols in dilute carbon tetrachloride solutions.

EXPERIMENTAL

The alcohols used in this work were either spectral or reagent grade. The substituted pyridines were obtained from Aldrich Chemical Company and were of the highest purity offered. The 4-cyanopyridine was obtained from Reilley Tar and Chemical Company. These chemicals were used without further purification.

Solutions used in determining the shifts of the O-H bands were 0.04 molar in alcohol and 0.50 molar in the base used. At these concentrations, peaks corresponding to both the free and hydrogen bonded O-H groups are present. The solutions of 4-cyanopyridine were saturated solutions in carbon tetrachloride at room temperature.

Infrared spectra were obtained in the range 3800-2800 cm^{-1} with a Beckman IR 8 Infrared Spectrophotometer using the procedure previously described (House and Cook, 1968). The free O-H stretching bands were quite sharp and easily located to within ± 2 cm^{-1}. The stretching bands of the hydrogen bonded O-H groups were broad and intense and maxima could be positioned to within only ± 4 cm^{-1}. The wavenumber shifts were thus determined to within about ± 6 cm^{-1}.

RESULTS

The positions of the O-H stretching wavenumbers of the alcohols hydrogen bonded to the bases and the shifts from the unbonded positions are shown in Table 1. The unbonded O-H positions were determined for

TABLE 1. Stretching Wavenumbers and Wavenumber Shifts in cm^{-1} for Alcohols Hydrogen Bonded to Pyridine (Py), 2-Methylpyridine (2-MP), 3-Methylpyridine (3-MP), 4-Methylpyridine (4-MP), 2,4-Dimethylpyridine (2,4-DMP), 3,4-Dimethylpyridine (3,4-DMP), 3,5-Dimethylpyridine (3,5-DMP), 2,6-Dimethylpyridine (2,6-DMP), and 4-Cyanopyridine (4-CP).

Base	pK_a	Methyl		i-Propyl		t-Butyl	
		Bonded	Shift	Bonded	Shift	Bonded	Shift
Py	5.20	3345	295	3359	265	3351	262
2-MP	6.01	3315	325	3327	297	3334	281
3-MP	5.66	3335	305	3344	280	3336	279
4-MP	6.04	3324	317	3330	293	3326	288
2,4-DMP	6.89	3305	334	3314	312	3315	299
3,4-DMP	6.54	3316	324	3320	304	3316	298
3,5-DMP	6.31	3317	322	3325	299	3316	299
2,6-DMP	6.68	3301	342	3322	302	3345	270
4-CP	1.90	3430	212	—	—	—	—

each spectrum so the positions are averages of at least eight independent determinations and are in excellent agreement with those of Motoyama and Jarboe (1966). Positions of the O-H bands of the unbonded alcohols are methyl 3641 cm^{-1}, i-propyl 3624 cm^{-1}, and t-butyl 3614 cm^{-1}. Also shown in Table 1 are the pK_a values of the bases used. These were taken from several sources and averages are shown in the table.

DISCUSSION

The spectrum of the solution of 4-cyanopyridine and methyl alcohol is particularly interesting. Three distinct peaks are observed at 3641, 3580 and 3429 cm^{-1}. The peaks observed appear to be due to the stretching of the unbonded O-H group, the O-H group hydrogen bonded to the cyanide group, and the O-H group hydrogen bonded to the ring nitrogen respectively. The corresponding wavenumber shifts are 61 cm^{-1} for the O-H bonded to the cyanide nitrogen atom and 212 cm^{-1} for the O-H bonded to the pyridine ring nitrogen atom.

One of the primary objectives of this work was to examine more closely the relationship previously reported between the base strengths of nitrogen heterocycles and their ability to cause the O-H stretching band to be shifted upon hydrogen bond formation (Tamres, *et al.*, 1954; House, 1967). A plot of the O-H stretching wavenumber shifts of methyl and i-propyl alcohols hydrogen bonded to pyridines *vs* the pK_a values of the pyridines is shown in Figure 1. As can be seen from this figure, the fit of the linear plot is very good. Although it is not shown in Figure 1, the data for t-butyl alcohol also give a linear relationship. The points for 4-cyanopyridine fit the linear plots very well although they fall off the scale of pK_a values shown in Figure 1.

When t-butyl alcohol is hydrogen bonded to 2,6-dimethylpyridine, the O-H shift is 270 cm^{-1}. This value was found several times and is smaller than that for some of the weaker bases. It appears that this

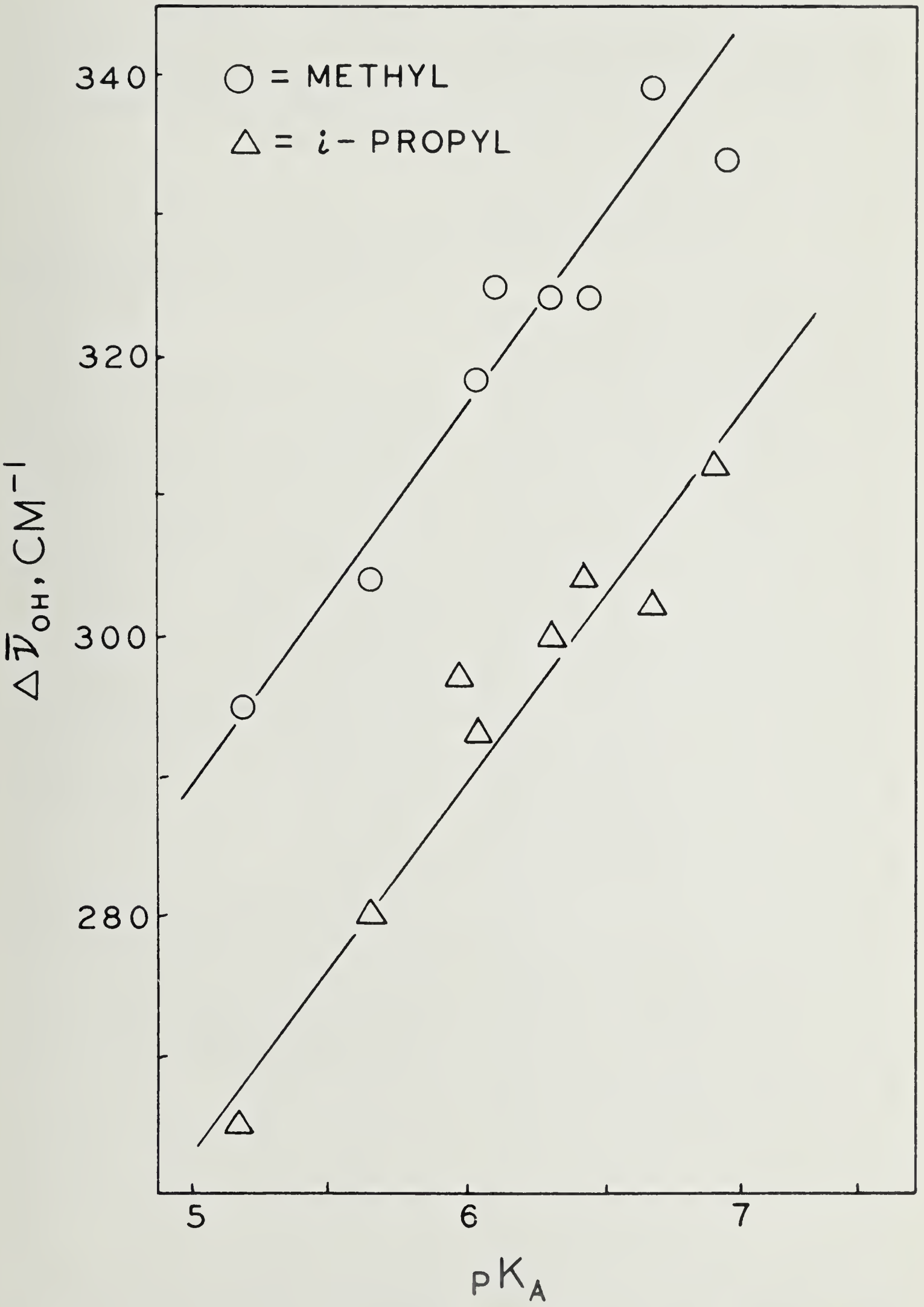

FIGURE 1. A plot of O-H stretching band shift *vs.* pK_a of the substituted pyridines.

smaller shift may represent some steric interaction of the t-butyl group with the methyl groups on the pyridine ring.

It is of some interest to compare the wavenumber shifts of the O-H group in CH_3OH found in this work with the O-D wavenumber shifts reported by Tamres, *et al.*, (1954) when the same bases are used. The ratio of O-H shift to O-D shift varied from 1.39 to 1.45 and averaged 1.42.

It has previously been shown that there is a linear relationship between pK_a values of pyridines and the formation constants of silver complexes of them (Sun and Brewer, 1967). A linear relationship has also been shown for the rates of displacement of the bases from some gold complexes (Cattalini and Tobe, 1966). It is thus apparent that if the basicity measured by the pK_a values is related to these parameters in this way, the hydrogen bonding tendency as measured by the ability to shift the O-H stretching band of a given alcohol is also linearly related to the formation and rate constants. When the formation constants reported by Sun and Brewer (1967) are plotted against the O-H wavenumber shifts of methyl alcohol, a linear relationship results. Similarly, a linear relationship is obtained between the O-H band shifts and the rate constants for the displacement of the bases from complexes of gold (III) reported by Cattalini and Tobe (1966). It is thus suggested that hydrogen bonding ability is a reliable measure of basicity for a similar series of bases.

Acknowledgments

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DEMONSTRATION OF DEFECTIVE MAZE LEARNING AND ABNORMAL ELECTROENCEPHALOGRAMS IN PHENYLKETONURIAC MICE

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ABSTRACT.—The effects of elevated levels of DL-Phenylalanine and L-Tyrosine in the diet of mice were studied. Litter mates of inbred albino mice were separated into experimental and control groups, equal in number and sex ratio. The experimental diets were supplemented with either 7% DL-Phenylalanine, 7% L-Tyrosine, or 3.5% of each of these amino acids. After three weeks, the animals were tested for the following responses: learning ability in a "T" maze, electroencephalogram patterns, body weight, and the presence of phenylpyruvic acid in urine samples. Deficient maze learning ability in experimentals, a characteristicly abnormal brain wave pattern, reduced body weight, and excretion of excess phenylpyruvic acid were noted in the experimental groups.

Phenylketonuria is an inherited metabolic disease appearing in the homozygous recessive condition and resulting from the inability to metabolize the amino acid phenylalanine. This inability is due to the inactivity of the liver enzyme phenylalanine hydroxylase which normally oxidizes phenylalanine to tyrosine. Phenylalanine then accumulates in the body and may reach near 30 times the normal level. The disease symptoms result from both the deprivation of the procursors necessary in the metabolic pathways, as well as, secondary effects relating to the inhibition of other metabolic pathways due to the concentration of phenylalanine.

The most serious effect of phenylketonuria is the mental defect. Untreated the development of the central nervous will be permanently retarded. Most phenylketonuriacs have low I.Q. levels, usually below 50.

Treatment of phenylketonuria depends on an early administration of a low phenylalanine diet. The symptoms can be arrested and sometimes reversed, and, with proper treatment patients have developed with near normal mental abilities.

It has been demonstrated that an excess of phenylalanine and (or) tyrosine in the diet of experimental animals can induce many of the physiological manifestations of phenylketonuria. Since metabolic disorders are often associated with abnormal electroencephalograms (Condon et al., 1954), abnormal patterns could be expected in phenylketonuria. Indeed both a decrease in learning ability (Auerbach, 1958) and alterations in electroencephalogram patterns (Cadell, 1962) have been observed in experimental phenylketonuria; also investigations by Gibbs (1941) found electroencephal-

ogram abnormalities in human phenylketonuria and described them with the outstanding features being high voltage, wave and spike discharges similar to, but more irregular than those of petit mal.

It is noted, however, that within the literature there is very little work demonstrating EEG patterns and learning ability in the same group of animals. Furthermore, there is some controversy concerning the time at which excess amounts of phenylalanine and (or) tyrosine must be administered (Woolley, 1964). Finally, the proposal that excessive serum levels of phenylalanine and (or) tyrosine might inhibit active transport of various amino acids during absorption in the intestine (Linneweh, 1963) seemed to indicate that physical measurements, such as body weight, might have significance, since a lowered body weight might result if essential amino acids were not properly taken up in the intestine. It would seem that if phenylalanine and tyrosine concentrations (independent variables) were increased at some level of maturation (independent variable) certain changes from control values in learning ability, electroencephalogram patterns, body weight, and chemicals excreted in the urine (dependent variables) could be expected.

Methods and Materials

Inbred strains of white mice were weaned at age 16 days, dividing the litter mates equally according to sex and number, into groups of controls and experimentals. The controls were provided with stock diet (Wayne Lab-Blox, 4% fat, 4.5% ash and fiber, and 24% protein- 0.83% tyrosine and 1.17% phenylalanine). Experimental group I

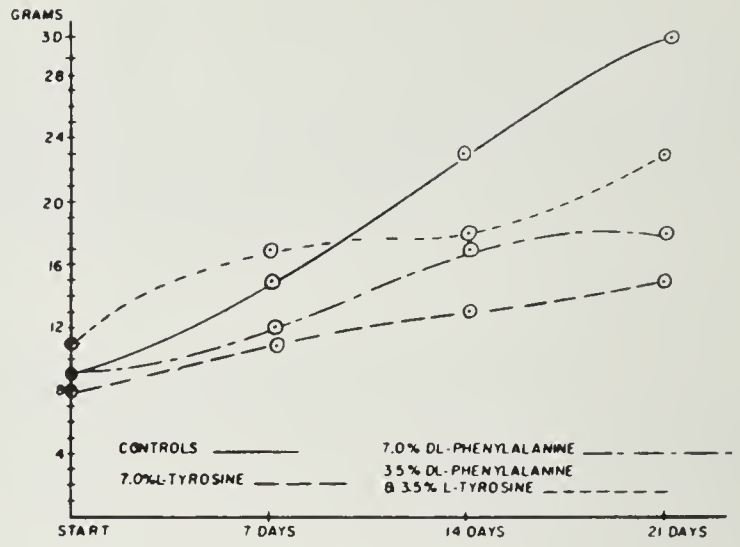

Figure 1. Weight during the period of experimental diet. Animals were placed on the selected diet at age 16 days.

received a standard stock diet with 3.5% L-Tyrosine and 3.5% DL-Phenylalanine added. Experimental group II received the standard diet with 7.0% DL-Phenylalanine added, while experimental group III received the standard diet with 7% L-Tyrosine added. During the developmental period (weaning to the time of testing comprising approximately three weeks) weekly measurements of body weight were made for both experimentals and controls. The amount of diet consumed was also recorded at the end of the growth period.

Maze Testing

When the animals were 6 weeks old they were tested in the maze (Fig 2).

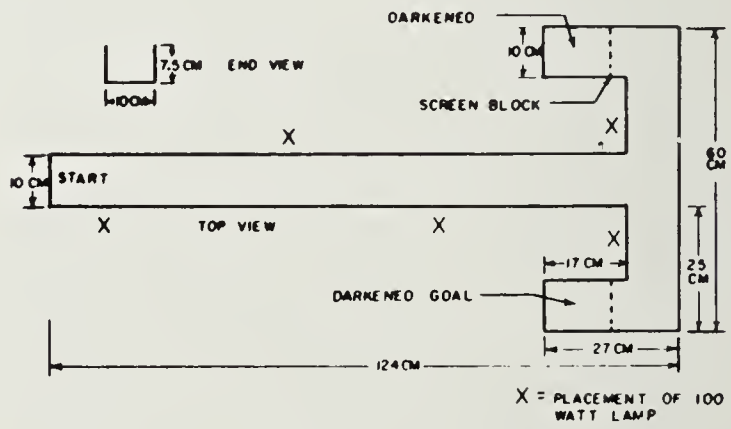

Figure 2. Diagram of maze used to test learning ability of mice. Material = 1/16" stainless steel.

Samples of urine were taken for analysis by Chromatographic methods. The maze was constructed of stainless steel, brightly illuminated, with one side providing a darkened hiding place, while the other side had a similar darkened hiding

place but the mouse was prevented from entering it by a wire mesh screen. The mouse entered the end of the channel and was given five minutes to acquire a spatial position habit consisting of a discrimination between the two equally darkened areas, one of which was closed to entrance. The stimulus was the bright light reflected from the steel surface into the non-pigmented eye of the albino animals. Rewards consisted of timed periods in the darkened hiding arm of the maze (goal). The maze was cleaned with 2% Lysol spray after the initial learning period to prevent scent from altering the results. For testing, the mouse was placed at the end of the channel and allowed to move down the channel to the cross of the "T", where a decision to move right or left was made. A positive response involved a movement toward the open darkened arm of the maze. The maze was cleaned between trials and process repeated ten times for each subject. Therefore random performance (lack of spacial habit acquisition) would have resulted in five positive responses in ten trials. Any number of positive responses above the value of five represented learning as defined in this experiment.

Electroencephalogram Method

In this experiment the purpose of the electroencephalogram was to establish an indication of general brain activity. Monopolar EEG patterns were taken on an E&M Hi-Gain Preamplifier-E&M Physiograph Model #4 combination. A grounded steel box enclosing the animal and shielded leads to the recording instruments were necessary to minimize 60-cycle interference. Nembutal (.02cc) was administered intramuscularly to reduce muscle artifacts, simplify EEG interpretation, and facilitate electrode attachment. Mice were placed in a rack which held them in a pronated position, with their head held firmly in place and which allowed a nasopharngeal electrode to be maintained in position. The other electrode to be maintained in position was attached to an electrically neutral region, the ear. EEG's were taken after the mice had been tested in the maze.

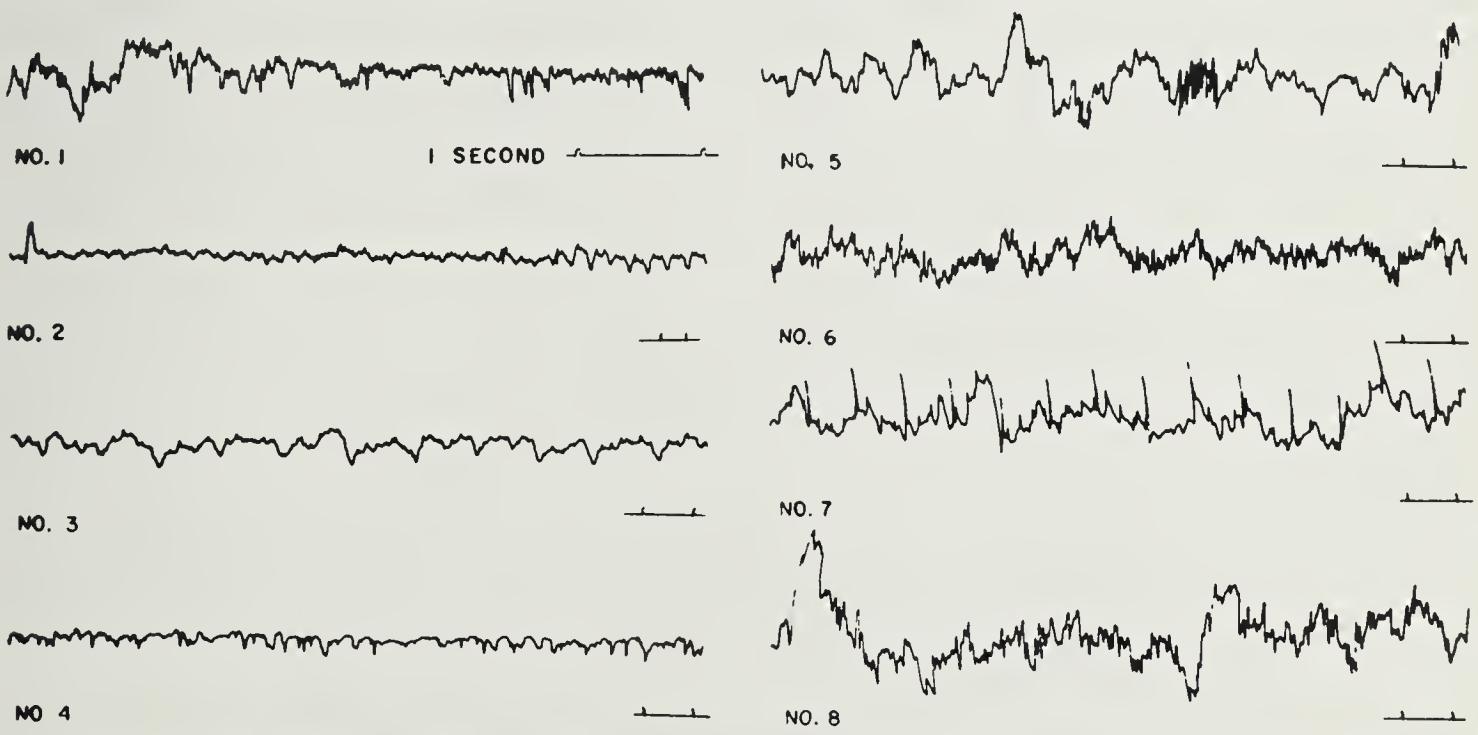

FIGURE 3. Characteristic electroencephalogram patterns shown with time intervals. No. 1-3—Controls; No. 4—Experiment 1; No. 5, 6—Experiment 2; No. 7, 8—Experiment 3.

RESULTS

The results of the maze learning test are shown in Table 1. Control animals were rather consistent in their performance with 7.5 (S.D. 0.4) positive responses in 10 trials. The group of animals receiving 3.5% DL-Phenylalanine and 3.5% L-Tyrosine demonstrated approximately the same results, while both groups of experimental animals receiving 7.0% L-Tyrosine and 7.0% DL-Phenylalanine respectively, showed signifi-

TABLE 1. Learning ability of mice as measured in maze learning test. Duration of diet: 3 weeks.

Treatment	No. of Mice	Maze Score
Controls	66	7.5±.4
3.5%DL—P+3.5%L—T	24	7.6±.8
7.0%DL—P	26	5.8±.6
7.0%L—T	25	5.5±.8

DL—P=DL—Phenylalanine
L—T=L—Tyrosine

cant reduction in maze performance. Analysis of control animals compared to the experimental diet groups consisting of 7% DL-Phenylalanine and 7% L-Tyrosine shows significantly reduced performance in the experimental group (p less than 0.28). There was no significant difference in performance of the control group when compared to the experimentals on the 3.5% DL-Phenylalanine plus 3.5% L-Tyrosine supplemental diet. Correlation between the group on the 3.5% DL-Phe plus 3.5% L-Ty diet and the other two experimental groups showed significant differences (p less than 0.27).

It was found that the representative EEG patterns of Experiment I (pattern number 4) appear essentially similar to the control EEG's (patterns 1, 2, and 3). In the groups receiving 7.0% L-Tyrosine (patterns 7 and 8) and 7.0% DL-Phenylalanine (patterns 5 and 6) respectively, characteristic large wave complexes were observed superimposed on a background pattern very similar to the controls. Ocassional irregularities of rhythm were noted as shown in example 5 of the EEG diagrams. The above observations represent qualitative clinical judgments and are not meant to imply quantitative analysis.

The average weight of the animals at age six weeks is shown in Table 2.

TABLE 2. Average weight of mice at six weeks. Animals were placed on diet when 16 days old.

Treatment	No. of Mice	Average Weight (grams)
Controls	66	30
3.5%DL—P+3.5%L—T	24	23
7.0%DL—P	26	18
7.0%L—T	25	15

DL—P=DL—Phenylalanine
L—T=L—Tyrosine

Controls were highly consistent with an average weight of 30 grams. The experimental groups all showed a reduced body weight with the lowest average being 15 grams for the 7.0% L-Tyrosine diet. Figure 1. shows the growth curves for the various experimental diets. All the curves are similar in slope for the first week. Beginning at 7 days the curves of the experimentals become flattened, while the growth curve for the controls has its greatest slope. The curves end with the controls significantly heavier than any of the experimental groups. This is in spite of the fact that equal amounts of diet per gram body weight were eaten by all animals. Analysis of experimental group body weights shows significant differences from control values (p less than 0.01). Furthermore the differences between groups receiving 7% DL-Phe and 7% L-Ty compared to the group receiving a mixture of 3.5% DL-Phe plus 3.5% L-Ty is significant (p less than 0.02).

TABLE 3. Relationship between the score in the maze test, excess phenylalanine, Tyrosine, and Phenylpyruvic acid in the urine, and irregular Electroencephalograms.

Treatment	Score in Maze	Chemicals in Urine	EEG Patterns*
Controls	7.5±.4	None	Regular
DL—P + L—T	7.6±.8	None	Regular
DL—P	5.8±.6	DL—P, L—T, PPA	Irregularities Present
L—T	5.5±.8	L—T	Irregularities Present

DL—P=DL—Phenylalanine L—T=L—Tyrosine PPA=Phenylpyruvic Acid

* Patterns of controls were studied to determine regularity of EEG; major deviations from controls as found in experimentals are explained in text.

The urine analysis (Table 3) shows that in the case of experimentals on the 7.0% L-Tyrosine diet, tyrosine was excreted in the urine. Animals on the 7.0% DL-Phenylalanine diet were found to have large amounts of phenylalanine, tyrosine, and phenylpyruvic acid in the urine. The appearance of phenylpyruvic acid and phenylalanine in the urine of all the animals receiving 7% DL-Phenylalanine was to be expected as such excretion has long been known to be a clinical result of such dietary treatment. Such excretion was observed in all cases of the 7% DL-Phe diet. Likewise the excretion of tyrosine in the experimental group receiving 7.0% L-Tyrosine supplement was to be expected and was observed. Tyrosine was expected in the urine of the experimental animals receiving 7.0% DL-Phenylalanine, since a large portion of the phenylalanine is transformed into tyrosine (Duncan, 1964) thus elevating serum levels to a degree exceeding the renal threshold. Such excretion was observed in 68% of all animals on DL-Phenylalanine 7% diet. (p less than 0.01)

Conclusions

It would seem from the results of the maze learning test (Table 1.) that excessive amounts of either DL-Phenylalanine or L-Tyrosine can cause significant reductions in learning ability in experimental animals. Excessive amounts of these metabolites also appear to affect the electroencephalogram pattern as noted by the characteristic differences in EEG observed between the experimental animals receiving 7.0% DL-Phenylalanine or 7.0% L-Tyrosine, and those receiving 3.5% DL-Phenylalanine plus 3.5% L-Tyrosine, or the standard diet. Table three summarizes the primary differences noted in maze learning, urine excretions, and EEG irregularities. It is noted when both DL-P and L-T are fed in excess, but in moderate amounts and in relative balance, that there is no significant change in any of the parameters measured when compared to the controls. However, when either DL-P or L-T is fed alone in great excess, the significant decrease in maze learning ability is accompanied by urine excretion of the compound or metabolite, as well as the appearance of abnormal EEG patterns.

The rather extreme reduction in body weight among the experimentals substantiates the hypothesis that physical factors such as body weight should be studied in experiments in-

volving special diets, and it is felt that the shape of the growth curves of the experimental animals from the seventh day to the fourteenth day on the diet may have significance. It is hypothesized that some time between the seventh and the ninth day serum levels of phenylalanine and (or) tyrosine reach levels which can inhibit absorption of essential amino acids in the intestine, thus causing a mild form of malnutrition. However, further research is needed to substantiate this point. No definite explanation can be offered at this time for the reduced maze learning or the abnormal electroencephalograms. However, if tryptophan absorption in the intestine, or across cell membranes were inhibited, this might result in a reduction in cerebral serotonin since tryptophan is a precursor for serotonin. Woolley (1964) feels that such a reduction from birth leads to decreased learning ability. Perhaps a similar reduction in slightly older mice will also reduce learning ability. Research is planned using specific agents (serotonin, its precursors, and inhibitors) which should help clarify this point.

Acknowledgments

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DISTRIBUTION AND ABUNDANCE OF BOG-INHABITING PSELAPHID BEETLES

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ABSTRACT.—The distribution and abundance of pselaphid beetle populations in a relict sphagnum bog are related to microhabitat variables and the ecology of each species. In northern Illinois, eleven pselaphid species with boreal affinities exhibited distributional patters related to stages in the bog sere. Each successional stage was characterized by unique moss species and microclimatological conditions comprising the microhabitats: the sphagnum mat, ericaceous shrubs, tamarack forest, and a swamp forest ecotone. Physical preferenda and predator-prey relations appeared to isolate sympatric beetle populations. Seasonal shifts in population between stages were affected by changing microclimate. Early successional stages were dominated by boreal species, where highest densities were attained with maximum development of the moss substratum.

Although the development of vegetational components through the various stages of bog succession is well known, e.g., Transeau (1903), Dachnowski (1912), Dansereau and Sogadas-Vienna (1952), and Heinselman (1963), little attention has been devoted to the faunistic relationships of the bog sere. It generally is accepted that the biota of relict bog communities in northern United States is of boreal origin (Braun 1928). Shelford (1963) concluded that there was no clear line of distinction between bogs of temperate regions and those characteristic of northern coniferous forests. Ross (1958) has shown that large number of insect species are abundant in both taiga and tundra and are almost entirely restricted to these major ecological formations. It is not unexpected, therefore, that certain boreal insect taxa have southern representatives which occur in bog habitats of temperate regions.

The Pselaphidae is a family of clavicorn beetles in the superfamily Staphylinoidea. Pselaphids are small beetles averaging about 1.5 mm in length. The world fauna consists of approximately 7500 described species (Park 1964) with the Nearctic complex being represented by some 500 species (Park 1958). The family is nearly global in distribution, with species occupying a variety of habitats to which they are often exceedingly specific (Park 1949). The basic, and probably ancestral, habitat is the mor-type organic layers of forest floors and their extensions or equivalents (Park 1942).

The studies of Transeau (1903), Dachnowski (1912) and other early botanists on bog development provided a classical description of physiographic succession. Between each successive community of the bog sere,

often within short and distinct spatial limits, vegetational components and physical characteristics change abruptly. Populations of bog-inhabiting pselaphid beetles occur within moss and litter microhabitats throughout all stages of bog succession, although various species exhibit predilection for habitats of particular successional stages. In the present investigation, combined field and laboratory studies were used to assess the dominant physical and biotic mechanisms which segregated these sympatric populations.

Bog Development

The Historical Aspect. Pleistocene glaciation covered most of Canada, northern United States, and mountain chains to the south. The last of the ice sheets, the Wisconsin (Fig. 1), had a significant effect upon the present topography and biota of glaciated areas. The biota of glaciated regions became established after a series of migrations related to ensuing climatic changes. With the renewal of milder climates and the consequent recession of the glaciers, displaced biotic associations spread in the direction of available habitats. Relict colonies — groups of plants and animals unlike those of the surrounding and dominant vegetation, but belonging to some distinct formation—remain as evidence of past climatic conditions (Braun 1928). As this sequential migration proceeded northward, boreal bogs characteristic of northern Canadian forests (Shelford 1963) remained as glacial relicts. These glacial hydrophytic relicts and their associated fauna have persisted in the more extreme habitats where the influences of the post-Wisconsin xerothermic period have not been strong enough to displace them. Replacement of surrounding vegetation by more southern types continued until present-day conditions were attained.

The Bog Concept. In eastern North America, bogs are frequent throughout glaciated territory and often occur to its southern limits. They are, however, most common within limits of the Canadian spruce-fir forests. Bog formations are present in several phytogeographical areas: the deciduous forest (Braun 1947, 1950), the tall grass prairie, the Canadian forest (Shelford 1963), and the taiga (Dansereau and Segadas-Vianna 1952). Conditions conducive to bog development occurred in closed depressions—kettle holes in glacial moraines, the quiet bays of lakes, or in calm situations in stream channels. The term "bog" has been used in ecological literature in many different senses. It sometimes has included marshes and swamps as well as bogs. Those who have used the term have emphasized various characteristics: vegetational (Carpenter 1938, Gates 1942), physical (Gates 1942, Potzger 1934, Transeau 1905), and physicochemical (Rigg 1916). Althought physical conditions often favor peat formation, Gates' (1942) definition of a bog as an area "vegetated by a flora in which peat-forming types of plants are particularly abundant" is followed in this text. In bogs the water table is at or near the surface and the soil is of organic (peat) origin, while swamps are characterized by a water table which is above (Potzger 1934) the surface and the soil is of either inorganic or humus nature.

Bog Succession. Bog succession has long been recognized in its broader aspects. Burns (1909) discussed early successional stages in bog lakes. The complete sequence from open water to mesophytic forests was described by Whitford (1901), Transeau (1903, 1905), Dachnowski (1911), and Dansereau and Sefiadas-Vianna (1952). Heinselman (1963) discussed a variety of successional processes beside that of the classical basin-filling pattern. The treatment by Dachnowski (1912) of vegetational succession in Ohio lakes and peat deposits is well known, and variations of this work have been reproduced in many texts. Fuller (1925) in an outline of Chicago area vegetation, listed the principal plant species associated with each of the major stages of bog sucession.

Marginal vegetation often will develop as a part of typical pond succession preceding bog development (Buchsbaum 1937). Such floating aquatics and emergent vegetation usually establish the initial stage of bog succession — the floating sphagnum mat. This mat forms around the anchorage of sedges and rushes, and often continues to propagate vegetatively (Rigg 1940). Expan-

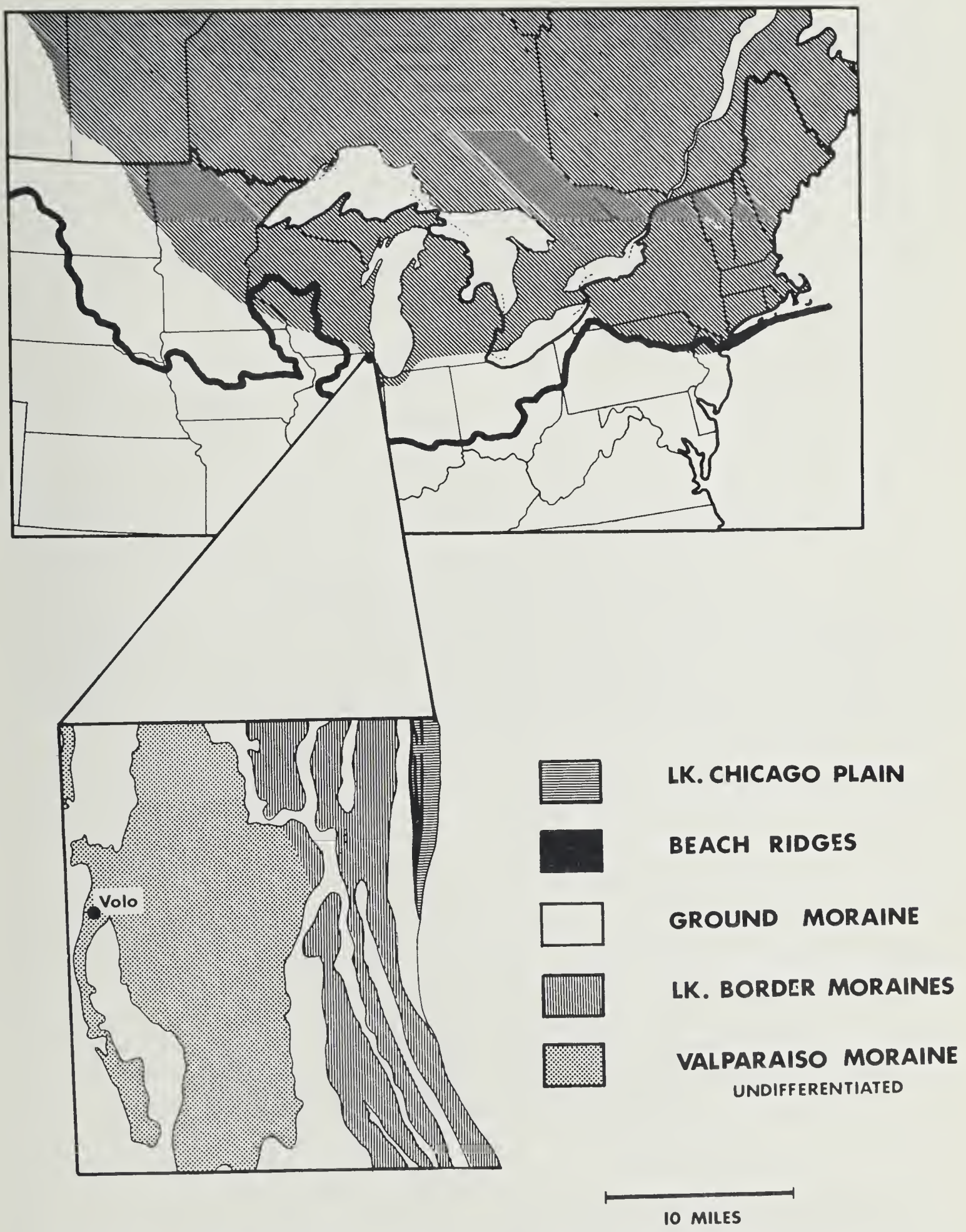

FIGURE 1. Southern limits (solid line) of Wisconsin glaciation in central and northeastern United States (after Hough 1958) and the general present-day distribution (shaded areas) of bog plants (after Transeau 1903). The distribution of bog-inhabiting pselaphid beetles closely approximates that of bog vegetation. Insert: morainic systems of Lake County, Illinois showing the location of the study area (modified from Fryxell 1927).

sion and centripetal encroachment of the mat proceeds over newly deposited layers of peat. The weight of accumulating materials causes a gradual sinking and an eventual filling to the bottom. Succession of vegetation is parallel to the filling-in of the water basin and its structure is parallel to the physiographic stages of development.

A consolidation phase follows with the establishment of a continuous sphagnum substratum which may vary in composition and structure. The differences in successive botanical features within a given geographic area is due primarily to fluctuations in water level. The sphagnum mat prepares a footing for the second stage—the ericaceous shrub zone. Sphagnum forms characteristic elevated hummocks around the bases of these shrubs. Shrubs initiate the transition to the next stage, through stabilization of the substratum by the binding action of their root systems, raising of the substratum by increased vegetative deposition, and modification of the physical environment. The next zone usually supports an association of tamaracks or tamarack-spruce in more northern localities. The substratum becomes noticeably drier, although infrequent pools may be found. Associated with reduced light intensities, a luxuriant fern growth is common. Sphagnum is no longer the dominant floor cover and other mosses and herbaceous vegetation appear. This stage is apparently a rather stable entity in bog succession, and it is not uncommon to find extensive bog areas covered by this single vegetational feature. Transitional woody associations often follow as the next stage; these are not restricted to bog sere, but are current subclimax types throughout their respective regions. The decline in edaphic influences eventually results in a merger with climax mesophytic communities of the surrounding area.

The Study Area

Sayer Bog, referred to in early literature (Waterman 1921, 1923, 1926; Kurz 1928), is now known as Volo Bog. This area was originally secured by the local Nature Conservancy and was placed under the administrative auspices of the University of Illinois. It is located one mile northwest of Volo, Lake County, Illinois, NE ¼ Section 28, T. 45N, R. 9E of the Grays Lake Quadrangle; alt. 229 m.

The Cary substage of the Wisconsin glacier extended west as far as central Minnesota and covered most of the territory north of the driftless area in Wisconsin. It reached its maximum southern extension in northeastern Illinois, northwestern Indiana and northern Ohio. Carbon-14 dating has placed the age of terminal Cary substage deposits at about 14,000 years (Hough 1958). The oldest and most pronounced morainic system established in northeastern Illinois during this period was the Valparaiso moraine, which averaged about 10 miles in width and marked the southern rim of the Lake Michigan basin. The bogs located in Lake County, Illinois (Volo Bog and others) occur in this Valparaiso drift (Fig. 1). This moraine is characterized by a clayey till of sand and gravel, frequently containing large percentages of calcium carbonate (Waterman 1926). The pollen sequence in Volo Bog (Artist 1934) follows the generalized pattern established for the Lake Michigan Region (Fuller 1935), with the postglacial sequence having been: *Abies-Picea*, *Picea-Pinus*, and *Pinus*-deciduous species. There is no definitive evidence to postulate an extensive postglacial tundra devolpment in this area.

Unusual smoothness (very few kettle holes or deep-set undrained depressions) characterizes the Valparaiso moraine in the Chicago Region. Most of the depressions of this drift have been drained into the river systems of the region, but a section on the edges of the drainage basins of the Fox and Des Plaines Rivers still contains a few poorly drained or undrained depressions, and it is in these that the bogs are found. Nearly all the lakes in this moraine topography have become swamps due to their initial shallowness (Bretz 1955). Since sphagnum was found to be dying out in most localities, Waterman (1921, 1923, 1926) believed this indicative of a move toward a drier habitat and that the bogs in northern Illinois were disappearing as the environment became unfavorable. Both Artist (1934) and Emerson (1921) failed to find sphagnum producing spores in this region and attributed this to unfavorable conditions brought about by alkaline waters, although the former reported no pH above 6.0 in sphagnum zones. In the course of this study, sporophytes of *Sphagnum teres* Angstr. have been recorded on consecutive years (1962-1963) in Stage II of Volo Bog.

Vegetational Associations

In the present study, the sucessional patterns in Volo Bog were subdivided into four basic stages (Fig. 2), which agree substantially with the floristic delineations of Fuller (1925). A complete floral anaylsis was not attempted. Emphasis was placed upon composition of the bryocophilous communities of the floor which comprised the pselaphid habitats (Table I). Only the charac-

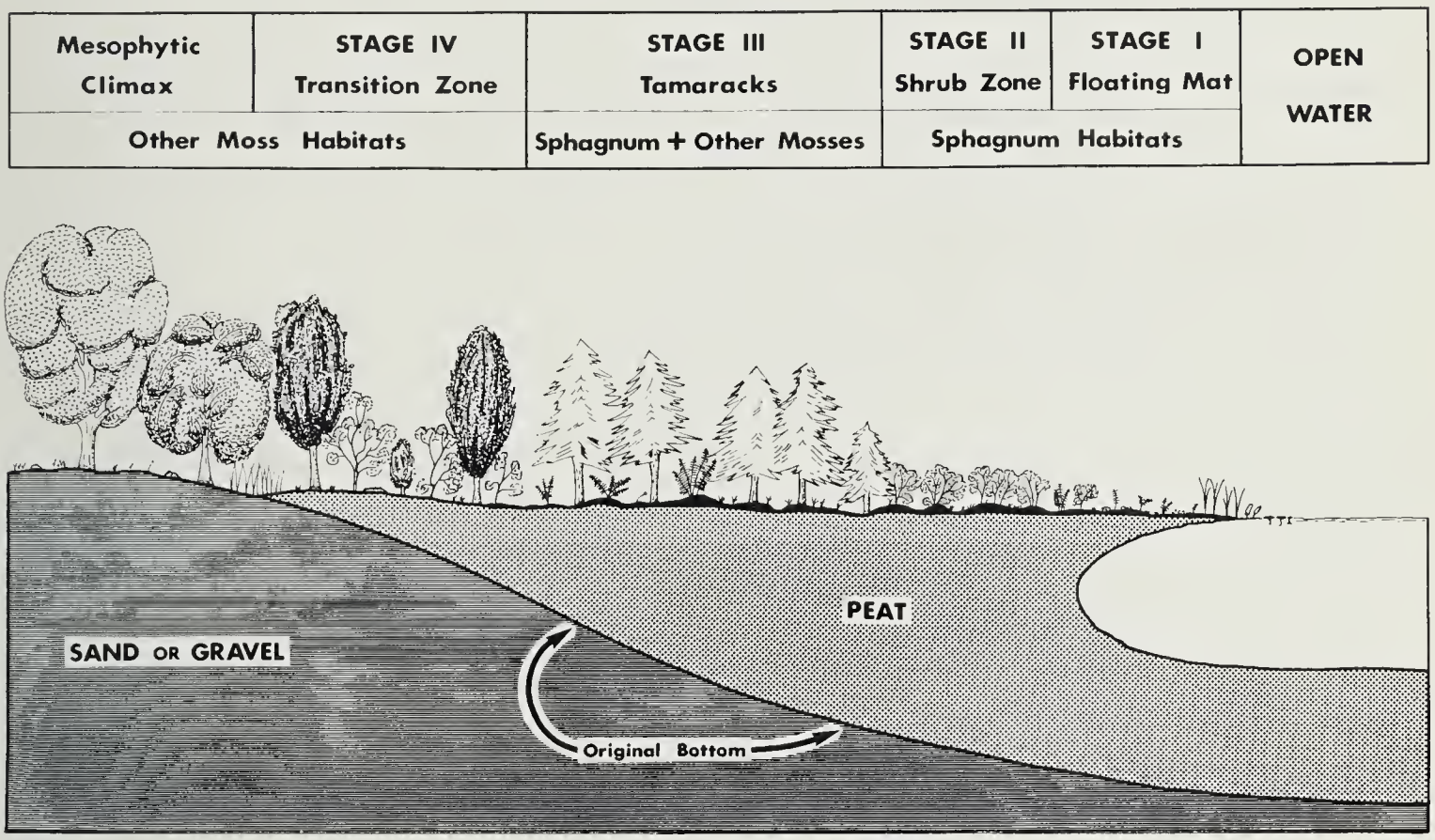

FIGURE 2. Diagrammatic cross section of bog succession depicting successional development at Volo Bog, with dominant vegetational features and seral stages. Areas represented by each association: Stage I, 0.12 ha; Stage II, 0.39 ha, Stage III, 2.85 ha, Stage IV, 14.12 ha.

TABLE I. Characteristic floral components of the successional stages of Volo Bog. Index species or those reaching their maximum density are indicated by an asterisk. All species in left-hand column are mosses.

STAGE I — FLOATING MAT—SEDGE ZONE

**Sphagnum cymbifolium* Ehrh.
**Sphagnum recurvum* Beauv.
Aspidium Thelypteris Sw.
**Carex* sp.
Juncus sp.
Menyanthes trifoliata L.
Typha latifolia L.

STAGE II — SHRUB ZONE

**Aulacomnium androgynum* Schwaegr.
Climacium dendroides (Hedw.) Web. and Mohr
Dicaranum scoparium Hedw.
Helodium paludosum (Sull.)
**Sphagnum teres* Anstr.
Tetraphis pellucida Hedw.
Calopogon punchellus (Salisb.) R. Brown
Drosera rotundifolia L.
**Oxycoccus oxycoccus* (L.) MacMillan
Pogonia ophioglossoides (L.) Ker.
**Rhus vernix* L.
**Sarracenia purpurea* L.
**Vaccinium corymbosum* L.

STAGE III TAMARACK ZONE

**Aulacomnium androgynum* Schwaegr.
Brachythecium sp.
**Calliergon cordifolium* (Hedw.) Kindb.
Helodium paludosum (Sull.) Aust.
Heterophyllium haldanianum (Grev.) Kindb.
.Mnium affine var. *rugicum* (Laur.) BSG
Plagiothecium sylvaticum (Brid.) BSG
Pohlia nutans (Hedw.) Lindb.
Polytrichum commune L.
**Sphagnum magellanicum* Brid.
**Sphagnum squarrosum* Crome
Ilex verticillata (L.) Gray
**Larix laricina* Koch
Onoclea sensibilis L.
**Osmunda cinnamomea*

STAGE IV TRANSITION ZONE (subclimax)

Aulacomnium androgynum Schwaegr.
Brachytecium sp.
**Calliergon cordifolium* (Hedw.) Kindb.
Helodium paludosum (Sull.) Aust.
Hetermophyllium haldanianum (Grev.) Kindb.
Mnium affine var. *rugicum* (Laur.) BSG
Mnium cuspidatum Hedw.
Plagiothecium sylvaticum (Brid.) BSG
**Thuidium delicatulum* (Hedw.) Mitt.
**Ilex verticillata* (L.) Gray
Iris versicolor L. (in atoll)
**Onoclea sensibilis* L.
Populus tremuloides Michx.

teristic vascular species of each stage are given to establish a comparative framework. The bryocophilous associations of bogs have been demonstrated to be reliable indicators of floor stratum development and existing microclimatic conditions of these habitats (Reichle and Doyle 1965). A more detailed discussion of the specific climatic influences and ecological factors characteristic of these microhabitats will be developed in subsequent sections.

The Bog Pselaphid Fauna

Pselaphids occurring in bogs are almost entirely bryocophilous, inhabiting the abundant moss floor cover in all sucessional stages. In eastern North America, bog pselaphid distributions follow quite closely the distribution of bog plants described by Transeau (1903) (Fig. 1). The relict bog biota does not extend southward beyond the Wisconsin terminal moraines to any appreciable extent and, in this pattern, reflects the historical relationship of these associations with glacial events. Pselaphid beetles occurring in bogs may be divided into two main groups: (i) index species restricted to bogs and comprising the dominant elements of the total population, and (ii) species occurring in later successional stages in niches ecologically equivalent to those of their normal swamp forest habitats. The index bog species appear to be glacial relicts of more extensive boreal populations (Reichle 1966).

In Table II are listed the ecological distributions of 22 common bog-inhabiting species in temperate North America. Thirteen species have been recorded from Volo Bog during the present study.

TABLE II. Bog-inhabiting Pselaphidae—a listing of their specificity and ecological distribution. Astersks indicate those species found at Volo Bog.

Species	Forest					Prairie		Bog, Swamp	
	under bark	leaf mold	log mold	tree-holes	ant nests	sod	ant nests	sphagnum bog moss mat	moss cover on swamp floor
Batrisodes furcatus (Brendel)	+	+	+	+				+	+
**Batriscodes golbosus* (LeConte)	+	+	+	+	+		+	+	+
Batrisodes nigricans (LeConte)								+	
**Batrisodes striatus psotai* Park								+	
**Bibloplectus* sp.								+	+
**Bythinopsis tychoides* (Brendel)								+	
**Decarthron abnorme* (LeConte)								+	?
**Decarthron defectum* Park								+	
Lucifotychus minor (LeConte)		+	+					+	
**Melba* (population B-11)								+	
Pselaphus bellax Casey								+	
**Pselaphus ulkei* Bowman								+	
**Reichenbachia borealis* Casey								+	
Reichenbachia corporalis Casey								+	?
**Reichenbachia puncticollis* (LeConte)								+	
**Reichenbachia scabra* Brendel								+	
Reichenbachia spatulifer Casey								+	
**Rhexidius canaliculatus* (LeConte)		+	+	+				+	+
Rhexius insculptus		+	+			+		+	
**Rybaxis clavata* Brendel								+	
Rybaxis transversa Fall								+	
Rybaxis varicornis Brendel								+	

Only three of the Volo species show a definite association with habitat types other than those characteristic of sphagnum bogs—*Batrisodes globosus*, *Bibloplectus* sp., and *Rhexidius canaliculatus*, are all immigrants in later stages of bog succession. *Both Batrisodes globosus* and *Rhexidius canaliculatus* are ubiquitous, and occupy a variety of different habitats. The remaining bog species are not dispersed indiscriminately throughout the bog sere but, instead, display a definite predilection for specific habitats. The nature of their relationships and the resulting distributional patterns within the bog sere form the subject of subsequent discussion.

PROCEDURES

During 1962 and 1963, weekly collections were made of pselaphid populations in each of the four stages of bog succession: sphagnum mat, shrub zone, tamarack fores, and transitional mesophytic forest (Fig 2, Table I). Two replicate moss samples (800 cm^2) were taken at random in each stage every week, following a transect from open water to the peripheral boundaries of the bog. At each sampling point, mean depth of the moss habitat was measured; this later permitted densities to be expressed per unit volume as well as unit area of habitat. Simultaneous with these collections, micro- and macroclimatic variables were measured in each stage. Field samples were then transported to the laboratory in large plastic bags for arthropod extraction.

Pselaphids and other microarthropods were extracted from moss using modified Tullgren funnels. Each funnel was con-

structed of galvanized metal 45 cm high, having a 71° slope, 30 cm top diameter, and 2.5 cm diameter opening at the bottom. Nine cm below the funnel top, a No. 4 screen was suspended inside on supporting brackets. The funnel was covered by a conical metal shade, containing a 60 watt incandescent light bulb. Moss samples were kept in funnels until dry—usually around 60 hours. Efficiency of pselaphid yield using this method exceeded 95% for all species.

Methods and Materials

U. S. Weather Bureau data for Cook County, Illinois, were used for reference macroclimate information. Maximum-minimum thermometers were used to record weekly temperature fluctuations within the woody vegetation cover. Recording thermographs supplied more detailed temperature values immediately above the moss floor cover. Temperature determinations within microhabitats were made with an electronic thermometer probe (Model TLB, Tri-Instruments). Field determinations of relative humidity within the moss habitats were made with an electric hygrometer indicator (Hygrodynamics Inc.). The narrow-range sensing elements (Model H-103) had an accuracy of ± 2.0% R. H. Continuous humidity readings above moss habitats were obtained from recording hygrothermographs. Moisture contents of mosses were based on 40°C air-dry Tullgren funnel weights.

Culture chambers for pselaphids were modifications of the plaster-charcoal technique (Rohde 1956). Polyethylene containers, 6 cm by 6 cm by 2 cm, with friction-top lids, incubated at 17°C were suitable for maintaining pselaphids, predators, and prey in the laboratory for many months. Experiments on survivorship and behavioral responses to environmental stresses were performed with similar cultures.

Results and Discussion

Seral Distributions. The preferred habitats of pselaphids in all successional stages were the well-developed moss growths. Forty additional 800 cm^2 random moss samples were taken between 15 July and 12 August, 1963, in each of the four successional stages, and the sample distributions of pselaphids were subsequently compared with Poisson distributions having the same mean (Table III). Since the sample data were a composite of several genera, frequency analyses also were determined for each species, if they were sufficiently abundant to enable statistical treatment. In all cases examined, the distributions did not deviate from that found for the composite populations. Therefore, it was assumed that the overall generic treatment did not mask contrasting lower-level species distributions. For each successional stage the mean (x) number of pselaphids per sample and the variance of each mean (s_x^2) are given (Table III). In the first three stages where

Table III. Comparison of observed and theoretical frequencies of pselaphids found in each of forty 800 cm^2 random moss samples, with calculations of X^2 for goodness-of-fit of data to a Poisson distribution. Collections taken: Stage I, July 15; Stage II, July 31; Stage III, August 7; Stage IV, August 12, 1963.

stage	mean number of individuals per sample	variance	χ^2	degrees of freedom	P
I	1.68	3.287	17.69	4	$P < 0.005$
II	2.10	3.785	23.03	4	$P < 0.001$
III	0.88	1.745	15.90	3	$P < 0.005$
IV	0.60	0.708	5.69	2	$0.05 < P < 0.10$

the distributions were nonrandom, the variances were greater than their means. The contagion can be resolved, therefore, as a "patchy" or "clumped" distribution of individuals rather than what is termed a "regular" distribution where the number of individuals in each sample closely approximates the mean and the variance approaches zero. There was also a significant increase ($P < 0.005$) in occupancy of sphagnum hummocks in younger successional stages (chi-square contingency test). The clumped distributions previously discussed may be interpreted by the fact that, although percentage moss floor cover was greater in younger stages, microhabitat heterogeneity (hummocks) resulted in clumped pselaphid distributions.

In Stage I the moss habitat was virtually uniform in floral composition, with two species of mosses predominating — *Sphagnum recurvum* and *S. cymbifolium*. The habitat, however, was not uniform in structure (Fig. 3). The typical floating mat of bogs was not represented at Volo; instead, the mat was impregnated with a dense stand of *Typha latifolia*. Sphagnum tended to form clumps at the bases of cattails, leaving either thinly-covered or inundated regions in between. This habitat was characterized by a pselaphid fauna of ten species (Fig. 4). *Reichenbachia borealis* attained its peak abundance in this stage, representing 60% of the mean annual pselaphid population. *Decarthron defectum* also reached peak distribution with 9% of the population. Although represented by only 12% of its total bog numbers in Stage I, *Bythinopsis tychoides* was still a dominant species with over 16% of the population in this stage. *Reichenbachia puncticollis* and *Decarthron abnorme* were species unique to Stage I habitats, although collectively they accounted for less than 2% of the population.

Stage II was characterized by a dense moss floor cover. *Sphagnum teres* was the dominant bryophyte in this zone at Volo, forming a continuous growth which was only occasionally disrupted by grasses or invaded by other mosses — notably *Aulacomnium androgynum*. Large dense hummocks of sphagnum formed about the bases of shrubs, attaining greatest development around *Rhus vernix*. This shrub has a characteristic basal-branching growth pattern which establishes an excellent foundation for hummock development. A clumped pselaphid distribution was correlated with this hummock development, with an increased frequency of pselaphids in the denser hummocks—a phenomenon which became even more striking as aggregations developed in late autumn and early winter. Stage II hummocks were inhabited by eight species of pselaphids (Fig. 4). Characteristic species and their respective mean annual percentage composition of the population were *Rybaxis clavata* (14%),*Pselaphus ulkei* (11%), and *Reichenbachia scabra* (4%). A major part of the population consisted of *Reichenbachia borealis* (34%) which progressively diminished in importance as the bog sere matured, although continuing as a conspicuous element of the fauna. *Bythinopsis tychoides* was rather uniformly distributed throughout the bog sere.

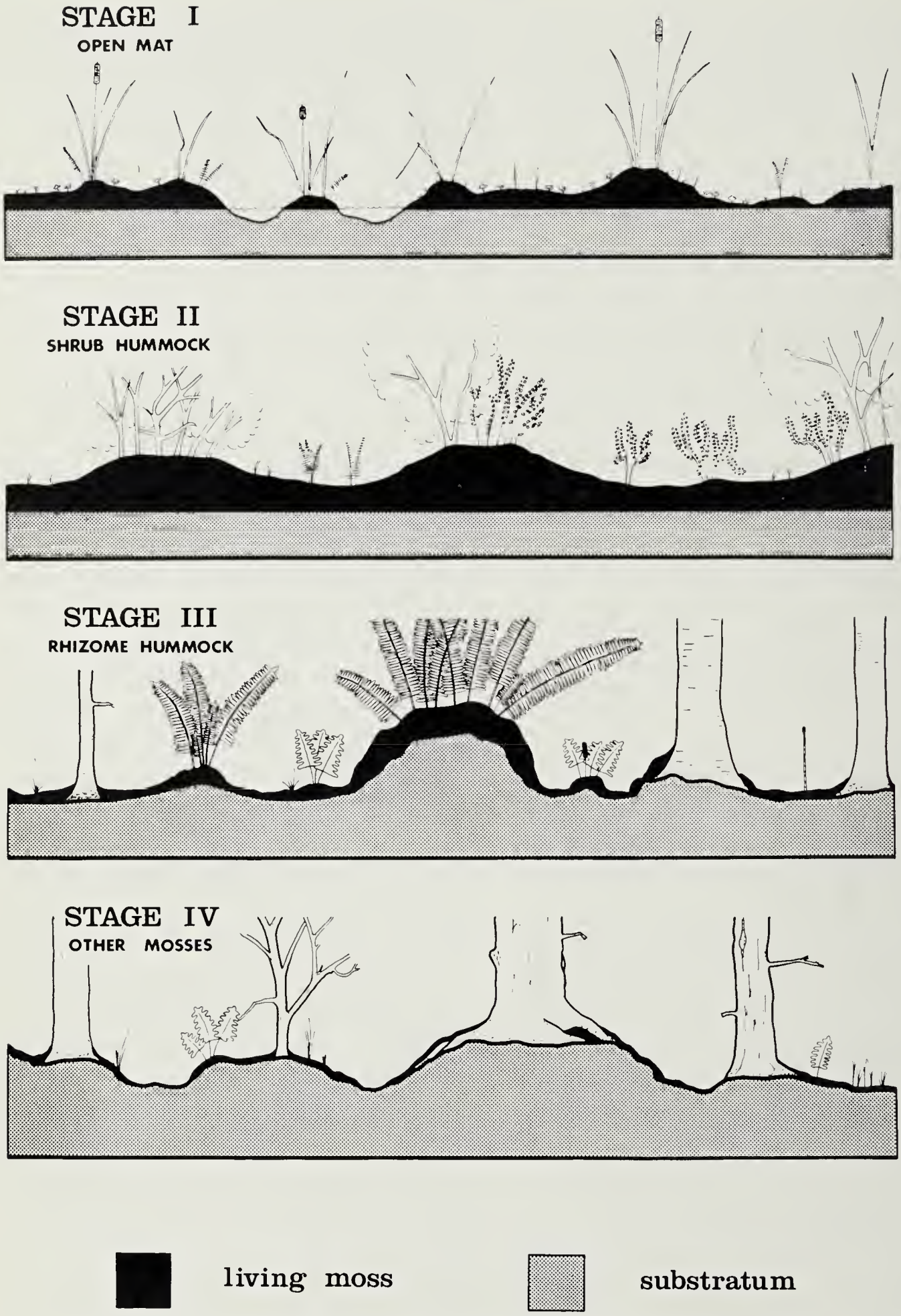

FIGURE 3. Comparative development of moss microhabitats at Volo Bog: Stage I, *Sphagnum recurvum* on the floating mat with early hummock formation; Stage II, *Sphagnum teres* in characteristic hummocks about the bases of shrubs; *Sphagnum squarrosum* and *S. magellanicum* persisting on fern rhizomes—intermittent regions, as in Stage IV, covered by a sparse growth of nonsphagnum mosses.

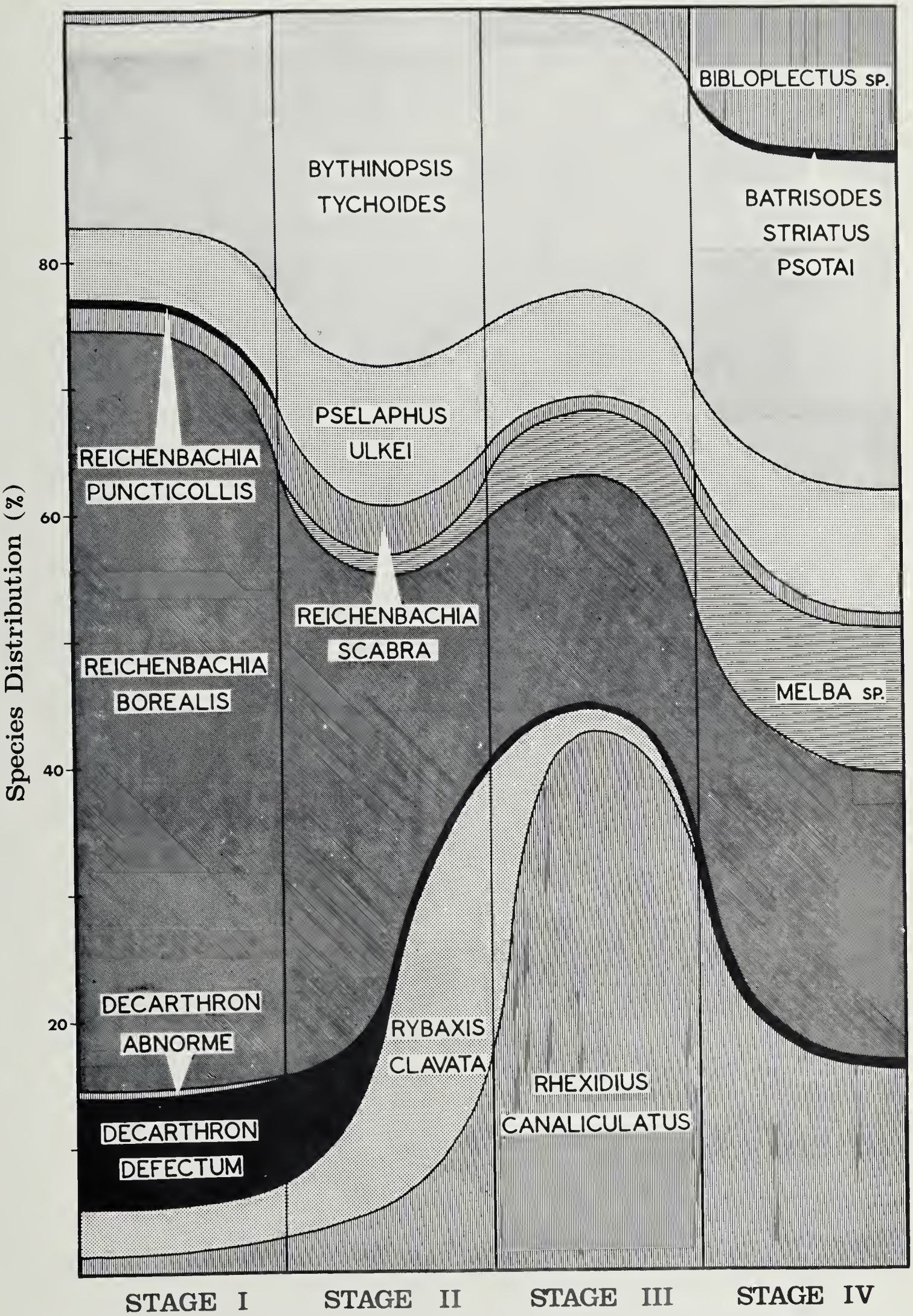

FIGURE 4. Seral distribution of pselaphid beetles at Volo Bog. Shaded areas show the percent of the annual population represented by each species. Species are listed in those stages where they reach peak distribution.

However, in Stage II *Bythinopsis* was most abundant and amounted to over 28% of the resident population.

In Stage III the floor cover of sphagnum (primarily *S. magellanicum*) diminished considerably, with growths usually occurring on the basal rhizome masses of the ferns *Osmunda cinnamomea* and *Onoclea sensibilis*. Numerous other bryophytes (Table I) occupied floor habitats in intermittent regions. Empirical observation would suggest a clumped distribution which, in fact, did occur. A greater percentage of the optimal sphagnum habitats were occupied, since fewer hummock habitats were available and the moss floor cover in between was poorly developed. In Stage III 70% of the hummock samples were occupied (containing 91% of the total fauna) compared to 62% occupancy in Stage II. The structural change in Stage III moss habitats was paralleled by a marked adjustment in the pselaphid fauna (Fig. 4). *Rhexidius canaliculatus* developed as a major component (43%) of the fauna, in association with the fern rhizome sphagnum hummocks. The populations of *Decarthron defectum* and *Rybaxis clavata*, characteristic of earlier successional stages, were no longer important aspects of the fauna. *Reichenbachia borealis* and *Bythinopsis tychoides*, although decreasing in percentage composition, continued as major elements of the fauna. Two new genera, *Bibloplectus* and *Melba*, make their appearance in the sere.

The nonsphagnum mosses of Stage IV presented a shallower but more continuous habitat. Although the microtopography of the area was heterogeneous, with elevations and shallow but extensive depressions with many exposed roots, the mixed-moss associes were nearly uniform in growth. For the first time in the bog sere, there was associated with this general consistency of the moss habitat a randomly distributed pselaphid population (Table III). This distributional pattern occurred only in the transition zone between true bog and mesophytic forest in a moss habitat devoid of sphagnum and its characteristic hummocks. In these transitional, nonbog habitats strong remnants of the bog pselaphid population still remained — *Bythinopsis tychoides* (28%), *Reichenbachia borealis* (25%), and *Rhexidius canaliculatus* (19%). However, sizable populations of swamp forest species *Melba* (12%) and *Bibloplectus* (13%) developed in the ecotone. *Batrisodes striatus psotai* appeared for the first time in this stage and, although a minor element of the fauna, has not been reported from other habitats and appears to be characteristic of late stages of bog succession.

Density. Density may be expressed as a function of unit area or unit volume of habitat. The objectivity of surface area or volume density measurement depends, to a large extent, upon the nature of the habitat being studied (Macfadyen 1952). In bog succession, not only did percentage floor cover of moss vary with maturity of habitat, but depth of moss growth also varied with hummock development (Fig. 3).

When the annual mean pselaphid abundance was expressed on an area basis (Table IV), Stage II appeared as the most productive habitat (49.74 indiv./m^2). Following in decreasing order were Stage III (40.79 indiv./

TABLE IV. Annual mean density of pselaphid beetles in each stage of bog succession expressed both per unit area and volume of moss habitat. Conversion from surface area to volume based upon mean moss depth in each stage.

SUCCESSIONAL STAGE	number of indiv./m^2 ± SE	$\bar{x}$ moss depth (cm)	number of indiv./10^3 cc moss
I	29.39 ± 4.84	6.0	0.49
II	49.74 ± 11.28	8.5	0.59
III	40.79 ± 10.85	5.0	0.82
IV	30.30 ± 9.35	3.0	1.01

m^2), Stage IV (30.30 indiv./m^2), and Stage I (29.39 indiv./m^2). This ordination approximated the degree of moss surface area coverage in each successional stage. However, when numbers of individuals per volume of habitat were compared, densities progressively increased through the sere (Table IV). Maximum densities (per unit volume) were attained in mature successional stages characterized by the greatest number of codominant species (> 10% of total population). In Stage IV, with the highest per unit volume density (1.01 indiv./10^3 cc), six species exceeded 10% of the total population, while Stage I (0.49 indiv./10^3cc) had only four codominant species.

Biomas. Dry weights of each species were determined from specimens frozen and dried in a vacuum desiccator (Table V). Although their range in size did not exceed 1 to 2 mm, considerable variation in mass existed between species. With the exception of *Batrisodes striatus psotai,* which occurred only rarely in Stage IV, larger species were associated with younger successional stages (Fig. 4, Table V). This observation subsequently will be discussed in relation to the role of the size of the moss interstices as a limiting factor in beetle distribution. Based upon annual population structure (Fig. 4, Table VI), and species weights (Table V), biomass estimates

TABLE V. Freeze-dry weights of eleven species of pselaphid beetles from Volo Bog (means calculated from pooled weights of 10 individuals).

Species	Sample size	$\bar{x}$ dry weight (μg per individual)
Batrisodes striatus psotai Park	5♂, 5♀	206.8
Bibloplectus sp.	5♂, 5♀	14.6
Bythinopsis tychoides (Brendel)	10♀	50.0
Decarthron abnorme (LeConte)	5♂, 5♀	110.0
Decarthron defectum (LeConte)	5♂, 5♀	56.0
Melba sp.	10♀	32.0
Pselaphus ulkei Bowman	5♂, 5♀	87.7
Reichenbachia borealis Casey	5♂, 5♀	126.7
Reichenbachia scabra Brendel	5♂, 5♀	92.9
Rhexidius canaliculatus (LeConte)	5♂ 5♀	34.5
Rybaxis clavata Brendel	5♂, 5♀	140.4

were calculated for each stage of bog succession. These estimates were based upon sampling of a total bog area of about 17.5 hectares, and did not include the total of some 19 ha included within the nature preserve, since a portion of the peripheral acreage to the south was occupied by a swamp community. Mean annual standing crop for each stage of bog succession was: Stage I, 3.01 mg/m^2; Stage II, 4.45 mg/m^2; Stage III, 2.52 mg/m^2; Stage IV, 1.88 mg/m^2. These estimates are based upon the average annual population structure, and do not include any of the seasonal fluctuations in numbers and mass or population shifts between different habitats. Maximum biomass occurred in earlier successional stages. Biomass also was correlated with degree of moss habitat development, and progressively decreased through the sere. This ordination is partially a result of a similar pattern in pselaphid densities, but was reinforced by size of species in each successional stage. Larger species were typically associated with the looser moss growths of earlier successional stages. Comparable biomass data for pselaphid populations in other ecosystems is nonexistent. Park, Auerbach and Wilson (1953) reported the density of a pselaphid population of an Illinois prairie to be 194,000 beetles per acre. Greatest densities in the bog sere were attained in Stage II with 104,000 beetles per acre.

Seasonal Population Fluctuations. Analysis of variance of the seasonal density of pselaphids in each successional stage revealed a significant

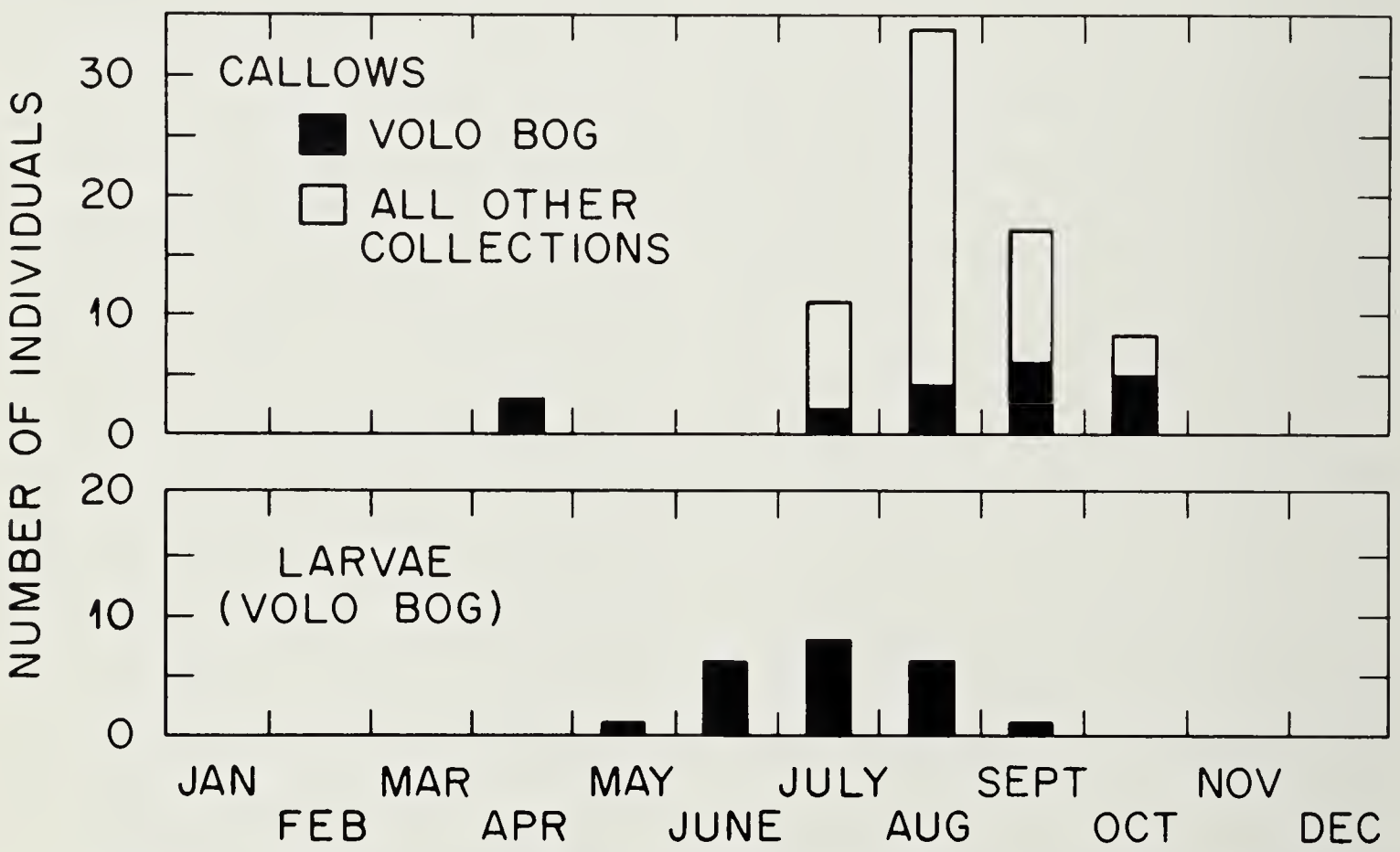

FIGURE 5. Frequency and temporal appearance of new pselaphid adults (callows) and larvae. Data from other geographic localities corrected for seasonal disparities and superimposed on the Volo time sequence. Values for Volo Bog are numbers of individuals per m^2 of moss habitat; other locality values are cumulative numbers collected and are comparable only in their relative temporal distribution.

($P<0.005$) effect of season on population size (Table VI). In early spring (April) highest densities occurred in hummock habitats in all stages. This was attributable to the rising water table following thaw and spring rains, which inundated the depressions and caused concentration of individuals in hummock "islands." A secondary population peak occurred in early autumn (September) and appeared to result from several factors. During the arid months of late summer and early autumn, the population shifted into the more protected moss habitats of Stages II and III. These habitats later served as winter hibernacula. This same interval included the period of emergence of new adults (Fig. 5)—a result of larval development which was probably initiated earlier that same year during the periods of high population density. Such seasonal fluctuations may be expected to vary over successive years due to varying climatic conditions. Low densities during December and January were most likely due to winter mortality and/or vertical movements into deeper peat layers. Even though no individuals were taken during January, the presence of mature individuals as early as February indicated that some adults did successfully overwinter.

The population distribution among successional stages revealed several density shifts with seasonal aspect (Table VI). During the winter months greater densities occurred in moss microhabitats in Stages I and II, which offered maximum depth stratification and climatic melioration during cold periods. Stage IV, with its shallow and sparse moss cover, had lowest densities. In spring the population moved out of hibernacula into the less protected habitats of Stages III and IV. Flooding of the floating mat, due to the rising water table, resulted in emigration from Stage I during this period. Lower densities in Stage I during late summer may have resulted from higher temperatures on the exposed mat, while drying out of the moss mat was an important factor causing lower densities in Stage IV. Stages II and III continued to vacillate during this period. With the less drying conditions of autumn, population density differences between stages were minimal. With

TABLE VI. Seasonal abundance (number individuals per m^2) of pselaphid beetles in each successional stage of Volo Bog.

Season		Stage I	Stage II	Stage III	Stage IV
Winter	$\bar{x}$	33.62	44.08	17.00	6.92
(Jan.-Mar.)	SE	22.03	26.98	12.06	6.22
Spring	$\bar{x}$	29.72	84.72	75.00	77.50
(Apr.-June)	SE	0.77	29.00	32.25	15.05
Summer	$\bar{x}$	24.48	42.71	44.27	25.00
(July-Sept.)	SE	1.38	12.77	14.33	7.16
Fall	$\bar{x}$	30.73	27.43	26.91	11.79
(Oct.-Dec.)	SE	3.17	12.90	5.14	6.04

the onset of winter climatic extremes, the population shifted into the protected hummock habitats of Stages I and II.

It would be premature to extrapolate these seral relations at Volo Bog to bog succession in other localities. Much will depend upon the quality of the fauna represented and the degree of development of the particular area. An important consideration is that this seral distribution shows only a secondary correlation with gross vegetational features, being a more direct result of paralleled development of moss microhabitats. Atypical bogs, or ones which have been disturbed, most often have been found to be characterized by a fauna related to the condition of the moss floor cover, rather than the above-ground vegetation cover.

Biotic Influences

Life Cycle. Little is known about the life cycle of pselaphid beetles. The nearctic fauna is composed of over 500 species, and the complete life history of none is known. Knowledge of the ecological requirements of developmental stages would be of great value in demographic studies of pselaphid populations. The larval stages of only two nearctic species have been described (Bőving and Craighead 1931). In the present study, larvae were extracted from moss, but attempts to rear these individuals were unsuccessful. Park (1947a,b) has conjectured about the absence of life history data, and has suggested several alternative means of obtaining information about the population dynamics of pselaphids.

Evidence for termination of the pupal stage was obtained from appearance of newly emerged adults (callows) in the population (Fig. 5). These records included nine bog-inhabiting species collected from 23 different localities over a three-year period (Reichle 1964). Callow adults taken from Volo Bog best represented the seasonal emergence of adults, since they were obtained from uninterrupted sampling throughout the year. The temporal occurrence of callows from all other localities was equated to the time sequence at Volo Bog by Hopkins' Bioclimatic Law (Graham 1952). An autumnal pulse of callows occurred at Volo which was reinforced by the frequency and temporal appearance of individuals in other localities. The records in April probably represented a spring emergence of lesser magnitude. Park, Auerbach and Wilson (1953) found similar biomodal peaks of callow abundance in their study of the pselaphid beetle population of an Illinois prairie.

Data for the bog population indicate a single annual generation: (i) adults overwinter within the frozen moss habitats of the bog; (ii) adult aggregations in spring and late summer could facilitate mating; and (iii) larval stages were present during the summer months. One annual pulse of larvae occurred, with maximum numbers appearing in July two months before peak callow densities (Fig. 5). No larvae were found in early spring which might have correlated with spring callows, making it unlikely that the latter represented a second generation. Furthermore, assuming equal developmental rates, a second vernal generation would necessitate larval development as early as mid-February when individuals

were inactive and the moss microhabitats were frozen. It is most probable that the three callows records in April represented individuals which pupated late the preceding autumn, overwintered as pupae, and emerged in the spring.

Predator-Prey Relations. Beetles of the family Pselaphidae are predators upon the microarthropod fauna of soil and litter habitats. Park (1932, 1933, 1947a) considered the feeding relations of pselaphids that were associated with ants. The feeding habits of five nonmyrmecophilic species were studied by Engelmann (1956). This study presents further information on the selective feeding behavior of six additional bog-inhabiting species. All observations were made on established cultures in transparent rearing chambers described previously. Feeding habits under laboratory conditions may vary from those existing under normal environmental situations, and this factor should be considered in interpreting results.

All positive (+) records of predation were noted (Table VII). The absence of feeding (—) was equally as significant, since this indicated that beetles starved without accepting these prey. Collembola served as the major food source for all bog pselaphid species, and were abundant constituents of the moss fauna in every stage of bog succession. Most bog pselaphids accepted only a specific group of prey. Entomobryid collembolans were eaten by most of the bog pselaphids, although *Bythinopsis tychoides* accepted only podurid and isotomid collembolans.

TABLE VII. Selective feeding behavior of pselophid beetles. The last five species are specific bog-inhabiting form.

	Collembola											
	Entomobryidae	Poduridae	Sminthuridae	Isotomidae	Acarina	coleopterous larvae	*Drosophia* larvae	Phoridae (larvae)	ant larvae	earthworms	tubifex worms
Ceophyllus monilis‡	+	—	—	—					+		
Tmesiphorus costalis‡	+	—	—	—		+			+		
*Cedius spinosus**	+	—	—	—		+					
*Bibloplectus sp.**	—	+	—	+							
Euplectus sp.†	—	+	—	+	+	—	—			+	+
Batrisodes globosus	+	—	—	+	+	—	+	+	+	+	+
Bythinopsis tychoides	—	+	—	+	—	—	—	—			
Decarthron defectum	+				—		—	—			
Pselaphus ulkei	+	—	—		—		—	—			
Reichenbachia borealis	+	—	—			—	—	—			
Rybaxis clavata	+				—		—	—			

‡ Park (1932, 1933).
* Engelmann (1956).
† Wagner (1962).

Batrisodes globosus, probably the most vagile pselaphid in temperate North America, exhibited the most generalized feeding behavior. *Bythinopsis tychoides* and *Reichenbachia borcalis,* with a congruent bog distribution, possessed different prey species. Prey specificity may well serve as an ecological isolating mechanism, removing these species from direct competition. While fluctuations in the abundance of some potential prey were noted between the different successional stages, there were no qualitative distinctions in prey between stages which might have limited pselaphids to particular habitats.

Relatively little is known about the food chains of bog arthropods. Cragg (1961) covered some aspects of the ecology of the soil fauna in English moors. Preliminary reports of a basic food cycle in sphagnum bogs have been made by Smirnov (1958, 1961). The basic energy flow pattern depicted in Fig. 6 for the terrestrial bog sere sumarizes the arthropod data from Volo. Sphagnum mosses and, to a lesser extent, higher vegetation are the basic contributors to detritus. Collembola and Acarina feed upon sphagnum through all stages of decomposition as well as the fungal growths supported by the detritus food base. Ptiliid beetles and insect larvae of several orders are fungal-feeders. Detritus also supports sizable populations of lumbricid and enchytraeid worms. Pselaphid beetles in turn prey upon a variety of these fungivorous and saprophagous invertebrates. The larger arthropod predators are primarily carabid and stayphylinid beetles and spiders which, subsequently, are the food of secondary vertebrate predators. The aquatic insect-nematoceran-ant food chain of Smirnov (1961) undoubtedly underestimated the role of Formicidae in all trophic levels of the moss community.

There are few records of predation on pselaphid beetles. The fact that other elements of the arthropod fauna are probably the major predators contributes to the paucity of such information. Staphylinid and carabid beetles have been observed to attack pselaphids in the laboratory and it is possible that these may be important predators in nature. Predation by salamanders has been reported by Park (1947a, 1964) and Park and Edgren (1950). Salamanders, however, have not been collected from the moss habitats at Volo Bog. Although stomach analyses of *Bufo americanus and Pseudacris triseriata triseriata* from Volo Bog have not yielded pselaphids, the presence of numerous microarthropods suggests that these toads and chorus frogs may be likely predators of pselaphids.

Insectivorous plants, when present, constitute a "predator" of considerable magnitude. The sundew *Drosera rotundifolia* was rare at Volo Bog, and its effect was of little importance. The pitcher plant *Sarracenia purpurea* was locally abundant, and accounted for a sizable toll upon the pselaphid population. From analyses of the contents of 17 pitchers eight pselaphids were recovered. Half of these individuals were *Decarthron defectum.* One *Reichenbachia borealis* was identified, while the remaining specimens were *Brachyglutini* (*Reichenbachia* or *Rybax-*

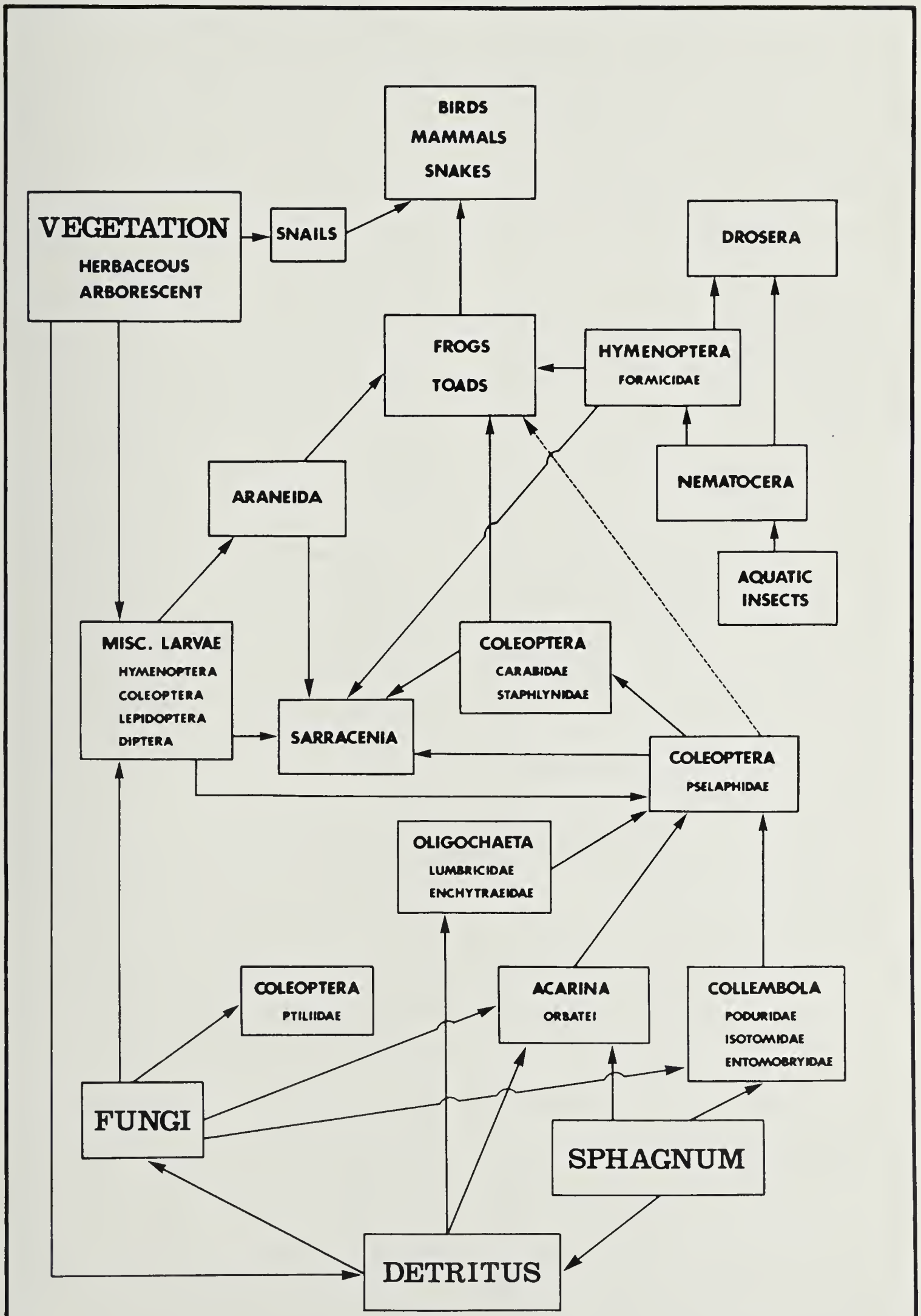

FIGURE 6. Principal pathways of energy flow in the bryocophilous community of sphagnum bogs.

is). The high incidence of *Decarthron defectum* trapped in pitchers may be correlated with its temperature preferendum. This species was characteristic of early successional stages where pitcher plants are most abundant, and its high temperature preferendum suggests stratification in upper moss layers in proximity to pitcher openings.

Parasitism. Parasitism may also play an important role in the population dynamics of a species by influencing densities, distributions, and intra- and interspecies competition. Several records of pselaphid parasitism can be reported for bog populations. Larval mites of the family Erythraeidae have been collected with mouthparts inserted through intersegmental membranes of *Reichenbachia spatulifer* and *R. borealis,* and scutacarid mites have been observed on *Reichenbachia borealis.* All of these mites are parasites of insects and other arthropods and show little, if any, host specificity. The erythraeids are parasitic in the larval stage and as nymphs, and adults are predators on other small arthropods.

The Laboulbeniales is a group of fungi which grow almost entirely on the external surfaces of insects. They are normally commensal and harmless to the host, living off the chitinous cuticula. Occasionally, with soft-bodied species, labouls may penetrate the body cavity with extensive rhizoidal processes. Since these fungi are transmitted from one living insect to another at times of direct contact, i.e., copulation, they tend to exhibit specific host relationships (Steinhaus 1949). Bog-inhabiting pselaphid beetles were infested by at least four species of Laboulbeniales. Comparatively high incidences of infestation occurred with *Autophagomyces bryaxalis* Thax. and *Pselaphidomyces pselapti* Speg. These two species commonly are found on the same individual, although *A. bryaxalis* appears to have broader host range. Two undescribed species — *Autophagomyces* sp. on *Decarthron defectum* and *Adelomyces* sp. on *Pselaphus ulkei* and *P. bellax*—were also present on bog species. Additional infestation records of bog-inhabiting pselaphid beetles exist (personal communication, R. K. Benjamin) : *Autophagomyces bryaxalis* on *Reichenbachia puncticollis; Autosphagnomyces* sp. on *Rhexidius canaliculatus; Pselaphidomyces pselapti* on *Reichenbachia puncticollis; Corethromyces elegans* on *Rhexidius insculptus.* The occurrence of these fungi on pselaphid beetles cannot be dismissed lightly. Their influence upon the dynamics of the bog population, however, must await further study.

Behavioral Patterns. Many terrestrial arthropods possess characteristic locomotory rates which vary according to environmental stresses such as predator-prey interactions, responses to climatic variables, and structure of the habitat itself. For the bog-inhabiting pselaphids, a positive correlation has been demonstrated between body size and locomotory rate (Reichle 1967). Such behavior may be important within the interstices of the moss habitats, since larger species are typically associated with looser sphagnum growths. In loose moss habitats the larger agile species could respond more swiftly to predators, prey, and climatic changes. In crevices of denser habitats, speed and

agility would be of lesser consequence, and the ability to maneuver within the moss interstices could shift the competitive balance in favor of smaller species.

Body contact with foreign surfaces often has an inhibitory effect upon locomotor activity. Low thigmokineses are typical of crevice-dwelling animals, and often are associated with their aggregative tendencies. Under natural conditions, such reactions will lead animals into sheltered places where they not only are protected from predators but also receive optimal climatic melioration. Bog pselaphids as a group exhibit thigmokinesis. In laboratory cultures, *Reichenbachia borealis* consistently localized ($P < 0.001$, chi-square contingency test) in corners and along side walls of chambers (Reichle 1964). There also was a significant ($P < 0.001$) preference exhibited for vertical surfaces. This response results from a negative geotrophism common in these species. Presumably, such behavior is associated with the stratified nature of their habitat and their preference for the upper living moss layers. It was common during field collections to find the majority of individuals localized near the surface of moss habitats.

The majority of species in the Pselaphidae are capable of flight. Flight activity is characteristically nocturnal, although this behavior is more typical of tropical regions and drops off sharply in temperate climates. Continuous light trap collections were maintained at Volo Bog from May to October 1962 without any records of pselaphids. This is contrasted by records of *Decarthron abnorme*, *D. defectum*, *Reichenbachia distans*, and *Brachygluta intricata* at light in early July in Wisconsin. Apparently under certain conditions, norturnal flights of bog pselaphids may occur. Although flights were not of sufficient range for migration between isolated bog localities, they could afford dispersal between habitats within the bog itself. The correlation of early summer flight activity with copulatory behavior and the appearance of new adults in the population, suggests a functional role of this behavior pattern in population dynamics.

Physical Environment — Microhabitat Factors

Temperature. Temperature extremes in bogs often exceed those of neighboring communities. Rigg (1947), in studies of air and soil temperature in a coastal sphagnum bog of the Pacific Northwest, found freezing temperatures in all months except August with temperatures consistently lower than those of the surrounding region. He attributed this to three basic factors: (i) setting of cold air in the bog depression, (ii) persistence of low temperatures in underlying peat from the preceding winter, (iii) cooling by evaporation and thermal radiation. Correlated with the successional development of bogs, there occurs a drop in substratum temperatures from the floating mat to the tamarack zone, and thereafter a general rise through later successional stages (Burns 1911). In Stage III, however, tamaracks have been found to exert their greatest modifying effect upon microclimate only during the summer months when in full foliage (Turnock 1955).

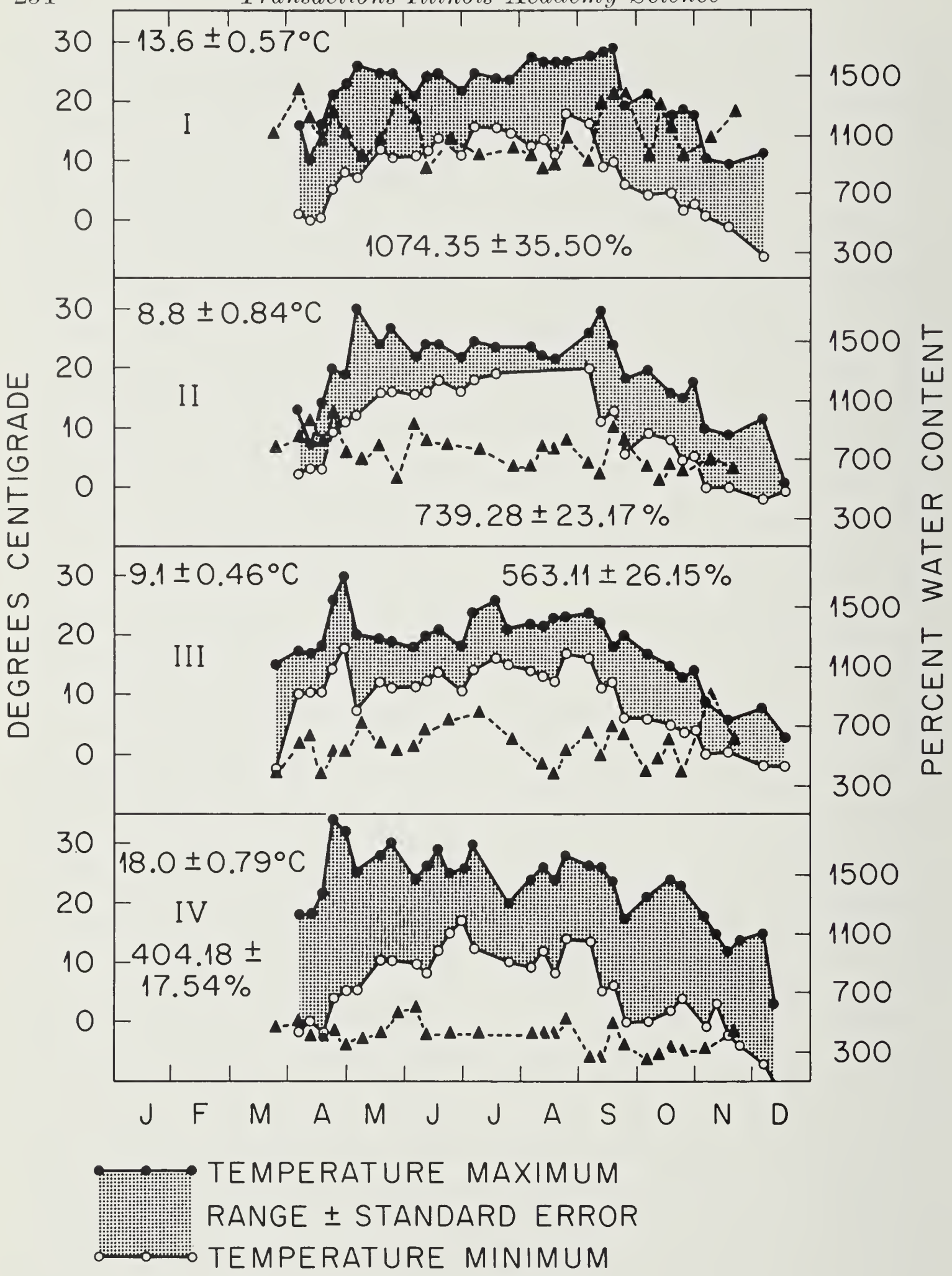

FIGURE 7. A comparison of the temperature regimes and moisture content of moss microhabitats in each stage of bog succession. Temperature curves show weekly extremes and weekly range. Values associated with each graph are (1) mean annual temperature range ± one standard error and (2) mean annual moisture percentage ± one standard error. Records taken beneath the moss mat: Stage I (6 cm), Stage II (8.5 cm), State III (5 cm), and Stage IV (3 cm).

Macroclimate temperature data for the Volo Bog region were obtained from U. S. Weather Bureau reports for the northwestern part of adjacent Cook County. Temperature regimes from March through December averaged 10.5°C with a range of from -20.5 to 35.0°C. In the Stage III tamarack forest at 10 feet, the temperature range for this same period was -30.5 to 32.0°C. Compared with regional conditions, not only did the bog have lower minimum temperatures but it also was subject to freezing temperatures during a greater portion of the year. Only between May and August were bog habitats free of freezing temperatures. Direct interception of incident radiation by the exposed sphagnum mat resulted in overall temperature regimes than those of the tamarack forest. Similar microclimatic differences also were evident between the moss microhabitats of different successional stages.

In analyzing the seral distribution of pselaphids in the bog, temperature conditions were characterized for the major moss habitats in each successional stage (Fig. 7). The annual extremes and the mean weekly temperature range ± one standard error were: Stage I, -5.5 to 29.0°C, 13.6 ± 0.57°C; Stage II, -2.0 to 30.5° C, 8.8 ± 0.84°C; Stage III, -3.0 to 30.0°C, 9.1 ± 0.46°C; Stage IV, -12.0 to 34.0°C, 18.0 ± 0.79°C. Sphagnum habitats in Stage I and the sparse moss habitats of Stage IV exhibited the greatest temperature variation. The more protected and denser moss growths of Stages II and III showed maximum temperature melioration.

Vertical temperature stratification within the respective moss habitats resulted in considerable melioration of above-ground environments (Fig. 8). Surface temperatures throughout the year showed a general decrease from pioneer to mature successional stages. The most abrupt temperature decline occurred between Stage I and Stage II. In Stage II evaporaiton in the absence of direct sunlight resulted in considerably lower temperatures. Shad-

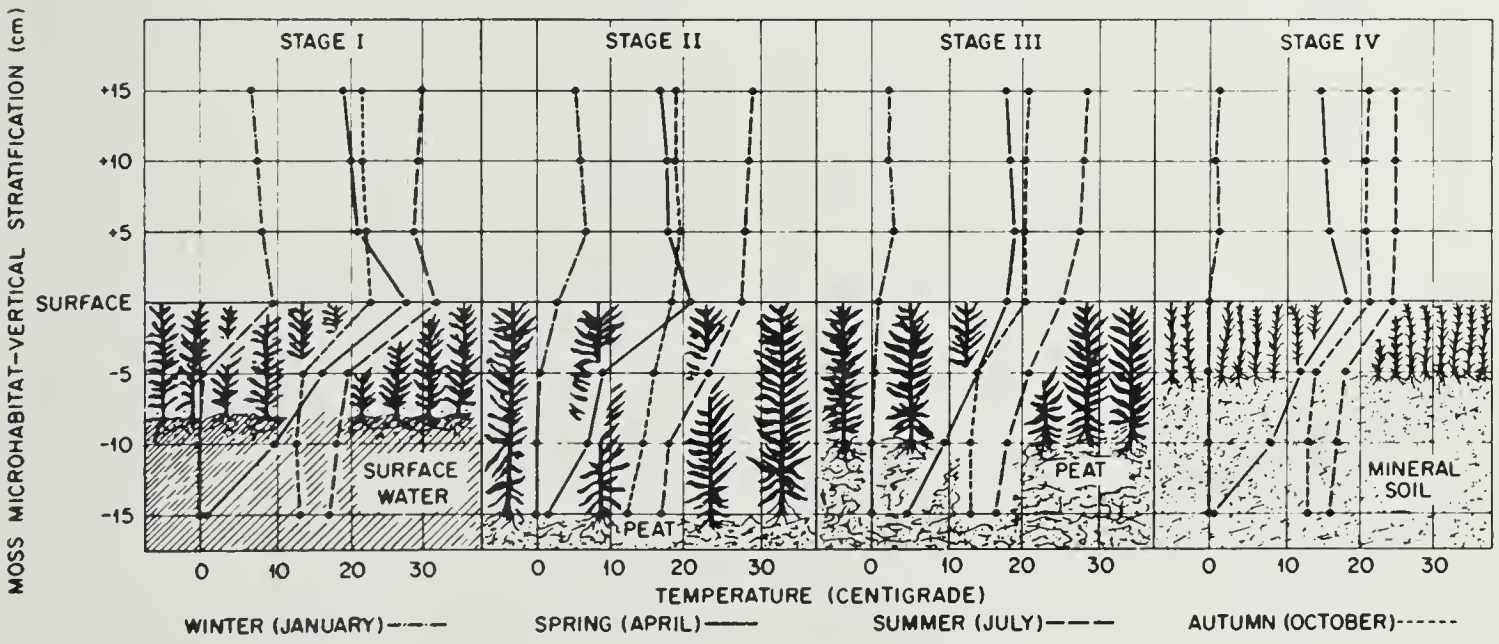

FIGURE 8. Vertical temperature stratification above and within successive moss layers for each season and successional stage. Growth habits of mosses in each stage are diagrammatically illustrated: first three stages sphagnum mosses, Stage IV nonsphagnum mosses.

ing by tree cover slightly enhanced this effect in Stage III. With increasing depth within the moss mat, obvious temperature differences between stages began to disappear. Seldom did temperatures above 20°C occur below 5 cm depth, and at 15 cm all localities had relatively uniform temperatures for any season. Norgaard (1951a) recorded only a 4°C range over 24 hours for summer temperatures at 10 cm depth in sphagnum, while temperature variations on the surface approached tenfold. With exception of the dormant winter season, however, pselaphid populations are generally confined to upper moss layers where temperature characteristics of moss habitats in different stages are distinctly different.

In the laboratory, July temperature preferenda were obtained for five bog pselaphids by Reichle (1967). The recorded temperature preferenda (Table VIII) correlated

TABLE VIII. Temperature and relative humidity preferenda of five bog-inhabiting inhabiting pselaphids (after Reichle 1967).

Species	temperature preferendum (°C) (mean ± standard error)	humidity preferendum (% R.H.)	locomotory rate (cm/min ± S.E.)
Bythinopsis tychoides	21.5 ± 0.81	> 95%	21.4 ± 0.68
Decarthron defectum	28.5 ± 0.55	> 95%	29.2 ± 1.07
Pselaphus ulkei	19.5 ± 0.86	> 95%	43.6 ± 1.00
Reichenbachia borealis	21.9 ± 0.99	> 95%	58.1 ± 1.30
Rybaxis clavata	28.3 ± 0.41	> 95%	67.1 ± 1.65

with temperature conditions of microhabitats where each species had peak distributions. *Decarthron defectum* and *Rybaxis clavata,* both characteristic of early successional stages, possessed the highest temperature preferenda (28.5 and 28.3°C, respectively). These preferenda corresponded to the higher moss surface temperatures in Stages I and II, and these species were rarely found in later successional stages. *Pselaphus ulkei* reached its peak distribution in the cooler moss habitats of late succession and, correspondingly, possessed the lowest temperature preferendum (19.5°C). *Reichenbachia borealis* and *Bythinopsis tychoides* were dominant species throughout bog succession. Their intermediate temperature preferenda were apparent adaptations to the broader temperature range encountered between different microhabitats. Whenever two species were codominants in a given microhabitat, e.g., *Reichenbachia borealis* and *Decarthron defectum* in Stage I and *Rybaxis clavata* and *Pselaphus ulkei* in Stage II, they possessed different temperature preferenda. This suggests that temperature stratification between microhabitats may be an isolating mechanism separating these sympatric populations. Similar temperature stratification of populations has been demonstrated for Danish bog spiders (Norgaard 1951a, b, 1952). In the case of

Reichenbachia borealis and *Bythinopsis tychoides*, with similar temperature preferenda (Table VIII) and congruent distributions throughout the bog (Fig. 4), each species had distinct feeding habits (Table VII). Different ecological niches would remove these populations from direct competition.

Year-round field collections of beetles and the postulated life cycle suggest that adults successfully overwinter within the interstices of the frozen moss mat. *Reichenbachia borealis* collected during the summer months and acclimated for 24 hours at 17°C showed a temperature preferendum of 21.9°C. However, *R. borealis* survived at temperatures between 4 and 27°C; $LD_{50\text{-}24\ hr}$ for temperature extremes were 1.5 and 33°C. Tolerance of extremely low temperatures, even in summer-adapted individuals, is indicative of the ability of these species to survive winter conditions. Collections of frozen moss will yield immobile individuals, which become active upon warming. Park, Auerbach and Wilson (1953) reported similar cases of cold hardiness for Illinois prairie pselaphids. The preferendum of the bog species, *R. borealis,* was only slightly below maximum tolerated temperatures, leaving a delicate compensation zone between preferred and lethal temperatures. Since high lethal temperatures are often approached in the upper moss layers, maximum lethal temperatures, rather than low temperatures, may be one of the critical factors affecting the distribution and abundance of the pselaphid population.

Moisture conditions. High humidities are characteristic of bog situations. Sheltered bog depressions have poor air circulation, and the saturated moss floor cover provides an excellent evaporating surface. Burns (1911) studied evaporation rates in a tamarack bog over a seven-week period in midsummer and gave the following relative values (no time units or evaporating surface areas stated): sedge zone, 321.7 cc; sphagnum under tamaracks, 275.0 cc; surrounding oak-hickory forest, 860.0 cc; adjacent field, 1056.0 cc. Since under given conditions the rate of evaporation is inversely related to moisture content of the air, these relative rates suggest high humidities within the bog proper. Although relative humidity of bog air is generally higher than for the surrounding region, it undergoes considerable daily fluctuation. Generally, near-saturated atmospheres develop during the night but these may drop by 50% during the day. Typically, the relative humidities within the interstices of sphagnum moss are high and undergo little fluctuation, even during the drying conditions of late summer. Moss habitats in the different successional stages vary in the degree to which humidity is accentuated, this being dependent upon relative depth and density of the moss growths (Table IX). The wetter mosses of pioneer stages have near-saturated atmospheres immediately beneath the surface, although microhabitat differences between stages are erased in lower moss layers. Stage IV microhabitats exert little modification upon humidity beyond that which can be accounted for through reduced wind velocities near the ground surface. Reduced air movement is an important fac-

TABLE IX. Relative humidity stratification in July for the four successional stages, showing comparative effects of moss microhabitats.

Levels	Stage I	Stage II	Stage III	Stage IV
15 cm above	60.0%	51.5%	52.0%	62.5%
10	61.5	54.0	52.5	63.5
5	63.0	56.5	56.5	64.5
0 surface	66.5	71.0	66.0	70.0
5	96.0	95.0	71.5	73.5
10	100.0*	100.0*	73.5	
15 cm below		100.0*	95.0	

* Supersaturation.

tor resulting in higher relative humidities immediately above and within moss habitats in all stages.

Reichle (1967) found that five different species of bog-inhabiting pselaphids consistently chose the most saturated atmospheres in experimental humidity gradients (Table VIII). Although all species preferred situations exceeding 95% R.H., they differed in the rates at which they responded. Presumably, such response times may give an indication of the relative tolerance of each species to adverse humidity stress. Ranking the species in order of increasing response time gave *Rybaxis clavata* < *Decarthron defectum* < *Pselaphus ulkei* < *Bythinopsis tychoides* < *Reichenbachia borealis*. Regression of response times on locomotor rates was not significant, indicating that these reactions were independent of locomotor ability. In the bog, optimum humidities are found in moss microhabitats in all stages except Stage IV.

Reichenbachia borealis and *Bythinopsis tychoides,* with greater tolerances of humidity extremes, were the only species of this group with significant distributions in the dryer nonsphagnum moss habitats of Stage IV. Conversely, *Rybaxis clavata* and *Decarthron defectum,* with rapid responses to changing relative humidities, were sharply limited to the saturated habitats of pioneer sucessional stages.

An important factor involved in the humidity relations of bog microhabitats is the moisture content of the substrata and mosses. Although there was considerable seasonal variation within each habitat type (Fig. 7), mean annual moisture contents were significantly different ($P < 0.01$). Moisture contents of mosses progressively decreased through the sere. Greatest differences occurred between the sphagnum moss habitats of Stages I through III. As sphagnum receded from open water and its growth habit became more elevated, the water content of its free haline cells decreased. These data corresponded to relative humidity values for these same habitats. A saturated moss mat and highest humidities occurred in pioneer stages, where ground water was abundant. The moss mat in Stage I received moisture from emersion in the bog lake as well as from precipitation. Although water content of the moss mat most quickly responded to pre-

cipitation, it was closely correlated to fluctuations in water level. Succeeding stages in the sere were characterized by elevated moss growths and drier moss mats. Mosses in Stages II through IV became increasingly dependent upon precipitation to maintain their moisture levels.

Other Physical Factors. Different vegetational components and moss habitats of the various successional zones interact to modify incident light intensities by as much as 60%. Greatest light reduction occurred within stratified layers of the moss microhabitats (Reichle 1964). At a 5 cm depth in all moss growths, light intensity was reduced to less than 20 ft-c. Associated with the low illumination levels of moss microhabitats was a characteristic negative phototaxis displayed by the pselaphid fauna. *Reichenbachia borealis* showed a significant ($P<0.01$) negative orientation to a collimated 70 ft-c light source. This is in the same range as illumination levels within the upper moss layers and well below light intensities on the moss surface. Similar behavior patterns have been reported for *Batrisodes globosus,* and have been suggested as selective mechanisms restricting species to protected habitats (Park 1947a). Although *Reichenbachia borealis* preferred low illumination levels, it was not entirely removed from photoperiodic influences. While high daytime intensities may be limiting, darkness and higher humidities may contribute to the nocturnal activity patterns characteristic of most pselaphids.

Wind velocity can often exert an indirect effect upon other climatic parameters. Accelerated evaporation rates due to air movement can severely limit the distribution of organisms with high moisture requirements. High evaporation rates and enhanced heat dissipation also will contribute to lower temperatures. Reduction in air movement through the various stages of bog succession was directly related to the degree of vegetational development (Reichle 1964). Air movement also was sharply reduced within the deeper layers of moss. Decreased circulation within the moss mat undoubtedly contributed the higher relative humidities within the interstices of the moss habitat.

Throughout the sere there was a successive development from a peaty substrate to mineral soils. Highest acidity was attained in early stages paralleling the development of sphagnum, although Stage I was influenced by the nearly neutral waters of the bog lake. Mean annual pH of the sphagnum zones were: Stage I, 6.5; Stage II, 5.1; Stage III, 6.1 (Reichle and Doyle 1965). When allowed to orientate on a substrate pH gradient from 3.2 to 9.0 (citrate-phosphate buffer), bog pselaphids displayed no predilection for substratum pH. Consequently, pH was not considered as a significant factor affecting their distribution in the bog.

The larger pselaphid species tend to be associated with habitats affording larger physical "living-space." Considerable variation in size of moss interstices occurred between microhabitats of the different successional stages. Sphagnum growths were characteristically looser in pioneer stages, with more compact

TABLE X. Interstitial volumes of sphagnum microhabitats in different stages of bog succession. Water-displaceable volumes measured from ten replicate 1510 cc moss cores from each stage.

	Stage I	Stage II	Stage IV
mean displaceable volume (cc) ± S.E.	1330.0 ±9.08	1396.5 ±5.48	1301.5 ±5.58
mean dry weight (g)	14.4	11.3	26.8

growths occurring in later successional stages. Significant differnces ($P<0.05$) in interstitial volumes between stages were demonstrated by displacement measurements (Table X). Water-displaceable volumes of sphagnum mosses decreased with successional development. Denser moss habitats were correlated ($P<0.02$) with greater moss dry weights per unit volume of habitat. Vertical stratification with increasing compactness with depth also occurred within any given moss habitat. This tended to mask overt successional distinctions between size of habitat interstices and size of pselaphid inhabitants. Nevertheless, it is noteworthy that the large species, *Rybaxis clavata, Reichenbachia borealis* and *Pselaphus ulkei,* were either limited to or reached their peak distribution in looser moss habitats of pioneer successional stages. The smaller genera, *Bibloplectus* and *Melba*, seldom occurred in sphagnum, but were characteristics of the more compact nonsphagnum moss and litter habitats of late bog succession. *Rhexidius canaliculatus,* one of the smallest species occurring in Volo Bog, was abundant in Stage III within the deep and compact sphagnum hummocks on fern rhizones. Interspecies competition may be manifest through maneuverability of species within the microhabitaa. Body size-interstitial volume adaptations could be associated with predator-prey relations, and could operate by segregating species to particular microhabitats.

SUMMARY

1. A relict beetle population (Pselaphidae) with boreal affinities, comprised of 11 species, was found to inhabit an isolated northern Illinois bog. All species were residents of moss microhabitats, and did not occur in similar habitats of adjacent communities. The distribution and abundance of this fauna throughout the bog sere was examined and correlated with changing ecological conditions of microhabitats. Microclimate, predator-prey relations, and behavioral patterns appeared to be the dominant factors isolating sympatric populations. Faunistic successional patterns paralleled the vegetational sequence, and were closely related to the structure and composition of the moss mat.
2. Succession at Volo, Bog, Lake County, Illinois, was classified as follows: Stage I — the floating sphagnum mat composed almost entirely of *Sphagnum recurvum,* which formed a compact mat exposed to environmental extremes

and changing water levels; Stage II — a shrub zone of heaths and *Rhus vernix,* which stabilized the peat substratum and facilitated characteristic hummock development of *Sphagnum teres*; Stage III—a tamarack (*Larix laricina*) forest characterized by a drier substratum and the appearance of nonsphagnum mosses, with *Sphagnum squarrosum* and *S. magellanicum* persisting only about the rhizones of the ferns *Osmunda cinnamomea* and *Onoclea sensibilis*; Stage IV—a transitional subclimax swamp forest, characterized by the absence of sphagnum and a diverse, but poorly developed, moss floor cover. Development of moss microhabitats was inversely related to overall succession and maturity of the respective bog communities.

3. Pselaphid populations of pioneer successional stages exhibited clumped distributions, with greatest densities occurring in optimal hummock habitats. In the absence of hummock formations in Stage IV, the population was randomly distributed. Greatest occupancy of hummock habitats occurred in early successional stages, where microclimates afforded greatest melioration of environmental extremes. Seral distribuitons were modified by seasonal aspect, though its imprint upon microclimates. Drier conditions of late summer resulted in a population shift into the more protected habitats of intermediate successional stages. Aggregations also developed here in winter hibernacula and in early spring, when rising water inundated all but elevated hummock habitats.
4. Population peaks in spring and autumn were influenced by these climatic phenomena and also by the appearance of new individuals in the population. Annual records of newly emerged callow adults and larvae suggested a life cycle with only one generation per year. Larvae appeared in late spring following adult aggregations; adults emerged in late summer and overwintered within the frozen moss mat.
5. Maximum species diversity was associated with pioneer successional stages and maximum structural development of microhabitats. Mean annual density was highest in Stage II (49.7 indiv./m^2) and only slightly lower in Stage III. Density progressively decreased through Stages III and IV. Biomass showed a similar relationship: Stage I, 3.01 mg/m^2; Stage II, 4.45 mg/m^2; Stage III, 2.52 mg/m^2; Stage IV, 1.88 mg/m^2. Biomass was positively correlated with the degree of moss habitat development.
6. With many sympatric species populations occurring within and between each stage of bog succession, ecological isolating mechanisms removed species from direct competition. Distinct microclimatic and physical conditions of habitats, temperature preferenda, and predator-prey relations segregated populations within the bog sere.
   a. Bog pselaphids exhibited behavioral responses favoring saturated atmospheres and

low illumination levels and consequently, a predilection for the dark, moist moss habitats of sphagnum bogs.

b. Each species possessed a distinct temperature preferendum characteristic of the microclimate in moss habitats where it reached peak distribution. In certain cases where several species were codominant, temperature preferenda and stratified temperature regimes within moss habitats served to further restrict species contact.

c. Larger species were associated with loose moss growths of early succession affording greater "living-space." Conversely, minute species were typical of the compact moss growths of late succession. Body size adaptations to moss interstices appeared to be related to predator-prey relations and interspecies competition.

d. Pselaphids were found to prey upon a variety of soft-bodied microarthropods, including Collembola, immature Acarina, and dipteran larvae, although each species was typically a predator of a specific group. Different feeding patterns, in lieu of climatic isolating mechanisms, served to separate the congruent bog populations of *Reichenbachia borealis* and *Bythinopsis tychoides* from direct competition.

7. Although most species were distributed throughout the bog sere, the majority had peak distributions in particular successional stages *Reichenbachia puncticollis, R. borealis, Decarthron defectum* and *D. abnorme* were index species of early (Stage I) bog succession. *Bythinopsis tychoides, Reichenbachia scabra, and Rybaxis clavata* were characteristic inhabitants of sphagnum hummocks in Stage II. *Rhexidius canaliculatus* was the dominant species in the dense sphagnum habitats in the tamarack forest. The transitional swamp forest zone was an ecotone between the true bog fauna and other lowland species. The typical species of this stage (*Batrisodes striatus psotai, Bibloplectus* sp., and *Melba* sp., seldom penetrated the extensive bog populations of earlier successional stages.

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THE ISOLATION OF ACTINOMYCETES FROM SOILS

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ABSTRACT.—Isolation of actinomycetes from forest and pasture soils was accomplished by using a soil dilution plate method. Two media and two fungal and bacterial inhibitors were compared to determine which was best for the growth and isolation of soil actinomycetes. The media used were Czapek's and glucose-egg albumen (Waksman, 1950). The inhibitors used were phenol, (Lawrence, 1956) and sodium propionate, (Cook et al, 1950). It was found that the treatment of soil dilutions with 1/150 w/v phenol allowed larger numbers of actinomycetes to grow than treatment of the medium with 0.4% sodium propionate. Moreover, fewer phenol plates than sodium propionate plates were overgrown by bacterial and fungal contaminants and greater populations of actinomycetes occurred on egg albumen medium than on Czapek's medium. *Streptomyces* and *Nocardia* species comprised the majority of the actinomycetes isolated under all experimental conditions. There was little difference in the variety of actinomycetes selected for by both treatment and media.

In the past several media have been used for isolating actinomycetes. The first man to publish on morphological and cultural studies of actinomycetes on synthetic media was Waksman (1916). Later Waksman (1950) published a list of synthetic media which have since been used by investigators for the growth and isolation of actinomycetes.

A survey of 21 commonly used synthetic media for the isolation of species of soil *Streptomyces* was made by Kuster and Williams (1964). They found that glucose and glycerol were the most frequently used carbon sources and that asparagine was the most frequently used nitrogen source. In addition they found that potassium nitrate, peptone and casein-starch were used with moderate frequency as nitrogen sources. The results of this comparative study on various media revealed that the same fungi occurred on all media, but that bacterial counts varied widely with media content (Kuster and Williams, 1964).

In a preliminary experiment for the present work Czapek's glucose-nitrate, glucose-egg albumen, and glucose-asparagine were compared. It was found that, in agreement with Kuster and Williams (1964), glucose-asparagine allowed excessive bacterial spreaders whereas Czapek's and gluecose-egg albumen did not. Consequently in future experiments it was decided to use Czapek's and glucose-egg albumen to the exclusion of glucose-asparagine. In their final experiment Kuster and Williams (1964) compared the five best media of their first experiment. They concluded that glycerol or starch as the carbon source and nitrate or casein as the nitrogen source were most suit-

able for isolating *Streptomyces*. In a similar experiment El-NaKeeb and Lechevalier (1963) found glycerol-asparagine to be superior among the conventional media they tested.

Cook et al (1950) were the first to experiment with the addition of an antagonistic chemical into actinomycete media. It was found that 0.4% sodium propionate incorporated into glucose-asparagine medium was optimum for actinomycete isolation and that concentrations above 0.8% were inhibitory to actinomycetes. Cook et al (1950) also found that although bacteria and molds would grow at the 0.4% concentration, the rate of growth was sufficiently reduced to allow for isolation of actinomycetes.

Lawrence (1956) compared the efficiency of sodium propionate and phenol for isolating actinomycetes from scaby potatoes and from soil. He found that maximum numbers of actinomycetes were obtained when phenol-treated suspensions were cultured on media adjusted to pH 6.5.

More actinomycetes developed on glucose-asparagine medium inoculated with phenol-treated potato scabs whereas on phenol-treated soil suspensions actinomycetes grew best on Czapek's medium. Lawrence (1956) found that phenol-treated soils were more efficient in reducing contaminants and permitted a greater number of actinomycetes to develop than when a medium containing sodium propionate (Cook, 1950) was used.

Methods and Materials

Pasture and forest soils were used for this experiment. The pasture soil was obtained from the Dralle Pasture on Old Monee Road in Crete, Illinois, and the forest soil was obtained from the Sauk Forest Preserve (Sherbert Woods) along Sauk Trail Road near Park Forest, Illinois. Thirty-two samples of both pasture and forest soil were taken from six inches below the surface of the soil after the litter had been cleared away. Each core was placed in a plastic bag and secured with a metal band. The cores were then brought to the laboratory and they were stored in the refrigerator until they were used.

Czapek's and egg albumen media were prepared and the pH of each medium was adjusted to 7.2 before sterilization by means of N/10 NaOH and N/10 HC1.

The procedures used for preparing the soil dilutions is tabulated below:

1. The 32 cores were thoroughly mixed and composited by passing them through a sterile sieve (6 meshes to the inch).
2. The composite soil was divided into two 25 gm samples designated as samples A and B.
3. The 25 gm sample was placed into a 500 ml graduated cylinder and sterile distilled water was added to bring it to a volume of 250 ml.
4. The 250 ml soil suspension was transferred to a sterile 500 ml Erlenmeyer flask and shaken hand over hand for five minutes.
5. Ten ml aliquots of the soil dilution were transferred through successive water blanks to obtain a 100,000 dilution.
6. One ml aliquots of the 100,000 dilution were placed on each plate.
7. The pH of the initial soil dilution was taken by means of pH paper.

The phenol treatment was performed by adding .65 ml of 88% phenol to the 100 ml final soil dilution. By doing this a 1:150 w/v dilution of phenol was obtained within the soil suspension.

Six plates per medium and per treatment were inoculated for each sample of forest and pasture soil and incubated at 27°C for two weeks. During the incubation period, the plates were observed for the general growth rates of the organisms on each medium and on each treatment. Finally, at the end of the incubation period the actinomycete, fungal, and bacterial colonies were counted and recorded.

The actinomycetes were enumerated only after they were isolated in tubes. Due to limited time, however, only two plates of organisms out of six plates per sample were given further cultural tests and microscopic examination. All of these organisms were identified to

genus by using a key from Waksman (1961). In most cases *Streptomyces* could be easily identified by macroscopic and microscopic observations. On the other hand the *Nocardia* were sometimes dicult to identify because of their similarity to bacteria and to *Streptomyces*.

No attempt was mad to identify *Nocardia* to subgeneric groups or to species. The *Streptomyces*, however, were classified to series according to Waksman (1961). Cultural and morphological characteristics were used to place the organisms in a series. The most important cultural characteristics for series identification was the ability of the organism to produce melanin. For melanin production the *Streptomyces* isolates were transferred from Czapek's slants to yeast extract-tyrosine slants and incubated at 27°C, and observed after two and four days of incubation.

Pigmentation other than melanin was also used as a taxonomic characteristic for placing *Streptomyces* into series. It was important to note the color of the substrate growth, the color of the aerial mycelium, the color of the spores, and the color of the pigment that diffused into the medium. For this experiment, therefore, all the color referred to in Waksman's key were determined according to Maerz and Paul (1950).

Results and Discussion

Czapek's and egg albumen media were first compared to determine which medium allowed the growth of the greatest number of actinomycetes. Tables 1 and 2 indicated that no significant differences were apparent between the two media. A comparison of the phenol versus sodium propionate treatment was made on forest soil samples A and B. The data in Table 2 showed no significant difference between the two treatments in terms of the total numbers of actinomycetes isolated from

Table 1. Total numbers of organisms enumerated from both phenol and sodilum proprionate treated Czapek's and egg albumen plates inoculated on pasture soil samples A and B.

	Actinomycetes	Bacteria	Fungi	Unknown
		Egg Albumen		
		Sample A		
Sodium Propionate	50	147	15	2
Phenol	128	5	1	3
		Sample B		
Sodium Propionate	51	329	3	14
Phenol	135	324	0	0
		Czapek's		
		Sample A		
Sodium Propionate	79	98	1	8
Phenol	90	3	1	0
		Sample B		
Sodium Propionate	51	107	3	2
Phenol	119	29	1	20

TABLE 2. Total numbers of organisms enumerated from both phenol and sodium propionate treated Czapek's and egg albumen plates inoculated with forest soil samples A and B.

	Actinomycetes	Bacteria	Fungi	Unknown
		Egg albumen		
		Sample A		
Sodium Propionate	25	113	4	6
Phenol	44	6	3	1
		Sample B		
Sodium Propionate	10	0	2	0
Phenol	24	4	1	3
		Czapek's		
		Sample A		
Sodium Propionate	12	39	4	9
Phenol	17	10	3	4
		Sample B		
Sodium Propionate	11	22	1	1
Phenol	19	31	1	1

both samples of forest soils. Sample A plated on egg albumen and treated with phenol, however, did have the greatest number of actinomycetes of the eight forest samples, and was close to being significantly different from the other samples.

A statistical comparison of isolates from pasture soil samples was also made. The data of Table 1 reveal that all the phenol-treated samples except Czapek's phenol Sample A had significantly larger numbers of actinomycetes, than had the samples inoculated on sodium propionate amended media. Phenol also proved to be a better fungal and bacterial inhibitor than sodium propionate for the isolation of actinomycetes from pasture soils. None of the phenol-treated samples were significantly different from one another, but both samples plated on egg albumen and treated with phenol had greater numbers of actinomycetes than the other pasture samples. It seemed, therefore, that the egg albumen-phenol was the best medium for the isolation of actinomycetes from pasture soil.

When both treatments are inspected, the data indicate more bacterial and fungal contaminants occur on the pasture soil samples than on the forest soil samples.

In this experiment, it was noted that the phenol treatments allowed fewer bacterial and fungal contaminants than the sodium propionate treatments with both media and both soil types. For example, pasture soil Sample A produced a total of 98

bacterial colonies on sodium propionate-treated Czapek's plates and it produced a total of only three bacterial colonies on the phenol-treated soil that was inoculated on Czapek's plates. Moreover the same soil sample produced a total of 147 bacterial colonies on sodium propionate-treated glucose-egg albumen plates and a total of only five bacterial colonies on glucose-egg albumen plates that were inoculated with phenol-treated soil. Pasture soil A produced only one fungal colony on sodium propionate-treated Czapek's plates that were inoculated with phenol-treated soil. Pasture soil sample A yielded a total of 15 fungal colonies on the sodium propionate treated glucose-egg albumen agar and only one fungal colony on glucose-egg albumen agar treated with phenol. Other soil samples gave similar comparative results between phenol and sodium propionate.

The ability to isolate the actinomycetes is as important a characteristic for distinguishing a good actinomycete medium as its ability to produce large numbers of actinomycetes. These treatments that most completely inhibited the growth of bacteria and fungi were also the treatments that proved most effective in the isolation of actinomycetes, i.e., phenol was more effective than sodium propionate treatments for isolation purposes.

Contaminants were very numerous on some of the sodium propionate plates and often completely overran the actinomycete growth. For exam-

TABLE 3. Series and genera of actinomycetes found from pasture soil samples A and B, plated on Czapek's medium and treated with sodium propionate and phenol.

Organism	Sodium Propionate Sample A	Phenol Sample A	Sodium Propionate Sample B	Phenol Sample B
Nocardia	4	4	5	13
Micromonospora	0	1	0	2
Unknown Organisms	1	2	4	3
Bacteria	4	0	5	2
Streptomyces melanin positive series				
Albus	2	0	0	0
Cinereus	1	2	0	4
Flavus	3	0	0	0
Violaceoruber	1	0	0	0
Streptomyces melanin negative series				
Scabies	1	0	1	0
Erythrochromogenes	4	2	4	2
Viridochromogenes	0	3	0	1
Streptomyces series unknown	1	1	2	2

ple, four plates and two plates out of a total of 43 sodium propionate plates were overrun by bacterial and fungal overgrowth, respectively. In contrast, no phenol plates were completely ruined by bacterial or fungal overgrowth.

Lawrence (1956) who compared sodium propionate and phenol in his experiments on the isolation of actinomycetes found that phenol was superior to sodium propionate in that both more actinomycetes grew out and in that the growth of fungal and bacterial contaminants was suppressed.

An important part of this experiment was to determine the types of actinomycetes that were selected for by the sodium propionate and phenol treatments. Since the soil samples had been thoroughly mixed, it was assumed that any differences in types of organisms isolated from the sodium propionate plates and the phenol plates were due to the difference in the selectivity of the two treatments.

When all soil samples were considered, it was found that *Streptomyces* occurred more frequently than any other actinomycete genus and that *Nocardia* occurred second most frequently. *Streptomyces* isolates were then identified to series after Waksman (1961). A comparison of the *Streptomyces* series that were isolated from each soil sample showed

TABLE 4. Series and genera of actinomycetes found from pasture soil samples A and B, plated on egg albumen medium and treated with sodium propionate and Phenol.

Organism	Sodium Propionate Sample A	Phenol Sample A	Sodium Propionate Sample B	Phenol Sample B
Nocardia	4	5	2	6
Streptomyces or *Nocardia*	0	0	0	2
Micromonospora	0	1	0	0
Unknown Organisms	1	2	2	2
Bacteria	2	1	2	0
Streptomyces melanin positive series				
Albus	1	2	2	2
Cinereus	0	4	1	2
Ruber	0	1	0	0
Violaceoruber	0	0	0	2
Streptomyces melanin negative series				
Scabies	0	0	0	4
Erythrochromogenes	1	4	2	2
Lavendulae	0	1	0	1
Viridochromogenes	0	3	0	3
Streptomyces series unknown	2	9	0	3

that a greater diversity of series were obtained from pasture soil than from forest soil. For example forest soil produced more organisms from *Cinereus* series than any ther series on both the sodium propionate and the phenol plates. The next most abundant series on the forest plates was *Erythrochromogenes*. On the other hand the most abundant pasture soil series were *Erythrochromogenes, Viridochromogenes, Albus,* and *Cinereus.*

Tables 3 - 4 list the types of actinomycete series and genera from pasture soil and tables 5 - 6 list the types of actinomycete series and genera from forest soil. Very little difference existed in the percentage of series types found on the phenol and the sodium propionate plates. The only difference was that the phenol treatment allowed more organisms per series to grow than the sodium propionate treatment. It was assumed, therefore, that the two treatments did not differ in their relative selectivity toward *Streptomyces* series. However, further experimentation should be done to determine the sensitivity of the various *Streptomyces* series to various levels of phenol and sodium propionate.

With both the sodium propionate and phenol treatments it was found that somewhat more than one-third of the *Streptomyces* series isolated from both pasture and forest soils were melanin-positive while the remainder were melanin-negative. Skinner (1938) and Waksman (1919) both found this same ratio of melanin-negative to positive *Streptomyces* series to exist in soils they sampled for actinomycete populations.

TABLE 5. Series and genera of actinomycetes found from forest soil samples A and B, plated on Czapek's medium and treated with sodium propionate and phenol.

Organism	Sodium Propionate Sample A	Phenol Sample A	Sodium Propionate Sample B	Phenol Sample B
Nocardia	0	2	3	1
Unknown Organisms	3	0	1	2
Bacteria	3	0	7	2
Streptomyces melanin positive series				
Albus	0	2	0	2
Cinereus	6	2	1	2
Streptomyces melanin negative series				
Erythrochromogenes	2	0	0	0
Viridochromogenes	0	1	0	0
Streptomyces series unknown	0	0	0	4

TABLE 6. Series and genera of actinomycetes found from forest soil samples A and B, plated on egg albumen medium treated with sodium propionate and phenol.

Organism	Sodium Propionate Sample A	Phenol Sample A	Sodium Propionate Sample B	Phenol Sample B
Nocardia	4	2	0	0
Unknown Organisms	0	3	1	1
Bacteria	1	3	0	2
Streptomyces melanin positive series				
Albus	0	1	0	0
Cinereus	2	0	5	4
Streptomyces melanin negative series				
Erythrochromogenes	0	2	0	0
Viridochromogenes	0	0	0	0
Streptomyces series unknown	0	0	0	2

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CERTAIN EFFECTS OF A METABOLIC INHIBITOR ON THE SEMINAL VESICLE-COAGULATING GLAND

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ABSTRACT.—Fructose and citrate concentrations in the seminal vesicles and coagulating glands of the male rat were affected by the use of fluoropyruvate, when injected. Fluoropyruvate may have blocked the transfer of hydrogen ions between pyridine nucleotide coenzymes.

Testosterone influences were noted in delaying the effects of fluoropyruvate on seminal fructose and citrate levels. It was assumed that the delay was due to competition of the excess androgen with the inhibitor for a common point on, or associated with, these pyridines.

Early work by Mann (1946, 1949) and Huggins and Johnson (1933) showed that the biosynthesis of both fructose and citrate in the mammalian secondary sex glandular secretions is controlled in some manner by androgenic steroids. The mode of action of these androgenic steroids has since been studied extensively, showing that they are involved indirectly in fructose and citrate formation by controlling rate-limiting enzymatic reactions (Talalay and Williams-Ashman, 1958 and Williams-Ashman et al., 1964). It was shown that steroid hormones were involved in the activation of hydrogen transfer between pyridine nucleotide coenzymes.

Avi-dor and Mager (1956) have shown an inhibiting influence by the use of fluoropyruvate. Considering pyridine coenzymes as a link between the accumulation of fructose and citrate in semen, an experiment was undertaken to determine the effect of androgens on pyridine transhydrogenations.

Ten animals were used for each interval of 15 minutes in the time study. Of the 10 animals in each group, 7 received fluoropyruvate injections and the remaining 3 were sham injected with an equal volume of physiological saline solution.

After establishing that seminal fructose and citrate levels were affected by fluoropyruvate, a procedure was designed to test the effects of testosterone propionate (Nutritional Biochemical Corp.) on the fluoropyruvate induced changes. Three groups of rats containing 25 animals each were given the following pre-treatment during a 10-day period prior to fluoropyruvate injections. Rats in Group I were castrated by scrotal incision after ether anesthesia on the first day of the 10-day period and received no further pre-treatment. Group II received daily injections of testosterone propionate at 5 mg per 100 grams body weight for the entire 10-day pretreatment period. Group III was treated similarly to Group II ex-

cept that the androgen injection dosage was increased five-fold to 25 mg per 100 grams of body weight.

After the 10-day pre-treatment period, 20 rats of each of the above 3 groups received injections of fluoropyruvate at the rate of 10 mg per 100 grams of body weight. Five animals were sacrificed at 30, 60, 90 and 120 minutes post-injection. The data from these 5 animals were compared with the remaining 5 control animals of each group, receiving only saline injections.

The results obtained from animals of Group II indicated the desirability of extending the experimental period of Group III by an additional hour. Ten extra animals were obtained and were given androgen treatment prescribed for Group III. This permitted sufficient animals to provide measurements at 150 minutes and 180 minutes for Group III.

Methods and Materials

Adult Sprague-Dawley male rats, ranging from 225-275 grams, were randomly selected and individually housed. All rats were given Purina Dog Chow and water *ad lib.*, except for 24 hours preceding sacrifice, when all food and water were withheld.

Seminal vesicles and coagulating glands, dissected out as a unit, were used as a reference gland for citrate and fructose utilization. The ventral prostate was not disturbed in order to avoid a prolonged recovery time. The glandular tissue was weighed and ground in a mortar, containing acid washed sand and 7 ml. of 10 percent trichloroacetic acid. Citrate was determined quantitatively by the method of Lindner and Mann (1960) with a Beckman Model B spectrophotometer set at 420 mu.

Analyses of seminal fructose concentrations were conducted on the supernatant obtained in the first part of the citrate procedure. The method of Lindner and Mann (1960) was used. Quantitative measurements of fructose and citrate were calculated on the basis of milligrams per 100 grams of wet tissue weight.

In the first part of the experiment, seminal fructose and citrate analyses were conducted on animals sacrificed at 15-minute intervals during the first 2 hours following an injection of fluoropyruvate. The dosage level of 10 mg per 100 grams of animal body weight was chosen, based on the work of Avidor and Mager, 1956).

Results and Discussion

The results of the time study on the effects of fluoropyruvate on seminal fructose and citrate appear in Figures 1, 2, and 3. The value shown at each time interval following treatment was based on the mean value for all animals of the group, with the standard deviation also indicated. All data, resulting from the various treatments were subjected to an analysis of variance. Comparisons of the mean concentrations of fructose and citrate were made at each time interval between 0 and 120 minutes post treatment. When the concentrations of fructose and citrate were compared at the different time intervals following treatment, significant changes in level of concentrations were noted. Significant chages in the level of concentrations were found in the normal androgen level groups of rats (Figure 1) and in the group of rats in which the androgen level varied (Figure 3), $P<.05$. No significant change in level was found in the castrate group of males (Figure 2), $.1> P<.3$.

Comparing the saline injected controls, in Figure 1, a marked fluoropyruvate effect was evident reaching a maximum in the case of citrate at approximately 75 minutes, preceded by the maximum negative effect on

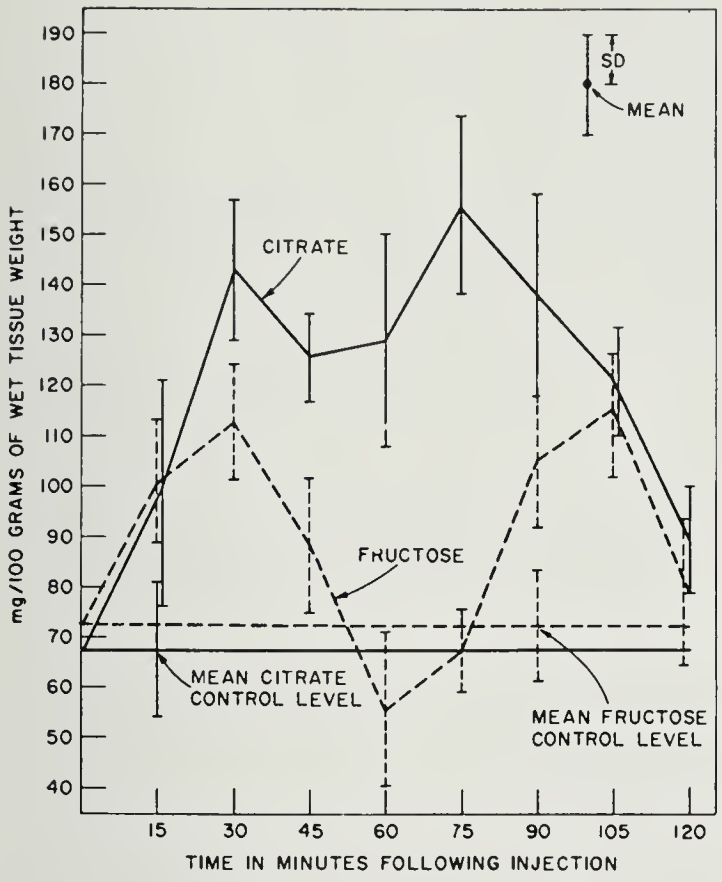

FIGURE 1. The effect of fluoropyruvate on the seminal citrate and fructose of animals at normal androgen levels.

fructose at about 60 minutes. Of special interest were the different effects on the concentrations of the two substances. A rise in citrate began at 15 minutes, with a lesser peak at 30 minutes. This was followed by a slight reduction in concentration, then another increase with the maximum at 75 minutes. Fructose concentration increased for about 30 minutes following injection then fell reaching its lowest point at 60 minutes. This low value of fructose concentration, which was approximately equal to those of the saline injected controls, was followed by a second increase reaching a maximum at approximately 105 minutes. The peak in citrate concentration was followed by a continuous fall, through the end of the second hour. At the end of the second hour the concentrations of both fructose and citrate again approached the values of the saline injected controls.

It is likely that the initial increase in the concentrations of both substances occurred before the inhibitory effect of fluoropyruvate became established. During this time a generalized increase in cellular activity in response to the stress produced by the injected fluorocompound might result in an elevated blood glucose level with a subsequent increase in seminal fructose (Mann and Parsens, 1960). Similarly, an increase in cellular activity could result in an increased rate of citrate synthesis. However, the initial increase in both substances was not observed in the saline controls and the possibility should not be overlooked that unexplained effects of the fluoro-inhibitor caused the initial increases.

The overall increase in citrate and the fall in the concentration of fructose can be interpreted on the basis of the inhibitory effects of fluoropyruvate on nucleotide coenzymes as they occur in the proposed coupled pathway of Samuels, Harding and Mann (1962). Inhibition of the proposed androgen mediated pyridine nucleotide transhydrogenations would result in less NADP+, which is necessary for the utilization of existing citrate stores. Citrate synthesis could be expected to continue with the resulting accumulation of citrate evident in Figure 1. The fructose level, according to this pathway, is determine by a controlled rate of synthesis, rather than a controlled rate of utilization, as in the case of citrate. Therefore, inhibition of the proposed effects of androgens on the necessary pyridines of this pathway would be expected to decrease the rate of fructose formation. The stores of fructose could have been metab-

olized rapidly by the glandular epithelium with the resulting low concentration shown at the 60 minute interval of Figure 1.

The fact that the peak of citrate concentration was preceded by the fall of fructose concentration and that the resumption of fructose synthesis precedes the fall of citrate can also be explained on the basis of the inhibition of androgen mediated pyridine transhydrogenations. Citrate utilization would be expected to resume only when fructose synthesis resumed with the concomitant formation of the NADP+ necessary for citrate breakdown. Similarly, citrate could be expected to accumulate when the rate of fructose synthesis was low.

The fall in the concentrations of both substances during the latter part of the 2-hour experimental period is considered to be the result of a lessened inhibitory effect of the fluoro-inhibitor. Such effects could arise through detoxification in the liver or to an increase in circulating androgens which would more effectively compete with the inhibitor.

The effects of different testosterone levels on fluoropyruvate inhibition appear in Figures 2 and 3. Analyses of the seminal fructose and citrate levels of the Group I castrated animals showed only trace amounts with no detectable change with respect to time following treatment (Fig. 2). During the 10-day interval between castration and treatment, insufficient androgens were available to maintain the integrity of the sex gland tissue as a whole. This was evident in the atrophied condition of the seminal vesicles and coagulating glands. Therefore, on these grounds, it would

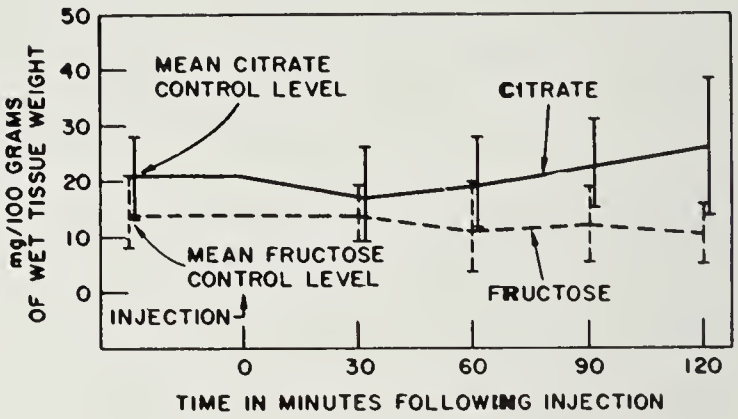

FIGURE 2. The effect of fluoropyruvate on the seminal citrate and fructose of animals castrated 10 days prior to treatment.

be erroneous to conclude that at very low androgen levels fluoropyruvate has no effect on seminal fructose and citrate levels. The lack of effect is thought to stem from a more basic change in physiological activity due to the low androgen level.

The values of the normal animals of Group II which received 5 mg of testosterone propionate per 100 grams of body weight each day of the 10-day pre-treatment period, were similar to those of the normal animals of Figure 1 with regard to both concentration and time.

More change was observed in the analyses made on the seminal vesicles and coagulating glands of Group III, which received testosterone propionate injections of 25 mg per 100 grams of body weight each day of the 10-day period (Fig. 3). The absolute concentrations of both substances were much higher, with both the peak of citrate accumulation and the minimum of fructose occurring about 30 minutes later than in the case of the animals of Figure 1 with normal androgen levels.

This apparent delay in the inhibitory effects of fluoropyruvate can be explained on the basis of more prolonged competition between the

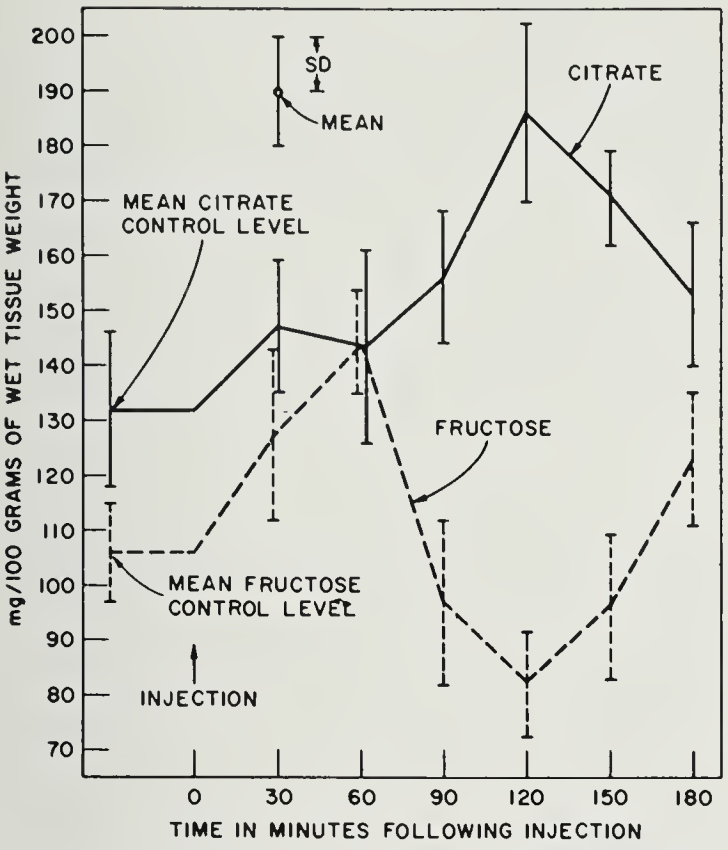

FIGURE 3. The effect of fluoropyruvate on the fructose and citrate levels of animals at a different androgen level.

androgen and the inhibitor for a common point on the pyridine coenzyme complex. Inasmuch as active competition between these two substances is suggested, the data again appear to be consistent with the inhibition of androgen mediated pyridine transhydrogenations in conjunction with the proposed coupled pathway of Samuels, Harding and Mann (1962).

CONCLUSION

The concentration of fructose and citrate in the seminal vesicles and coagulating glands of the rat were found to be affected by fluoropyruvate. Acting as an inhibitor, this compound may have blocked the transfer of hydrogen ions between pyridine nucleotide coenzymes. The results obtained were consistent with the inhibition of a proposed mediation of hydrogen ions between these pyridines by androgens.

A high level of testosterone delayed the effects of fluoropyruvate on the seminal fructose and citrate levels. It is assumed that this delay occurred due to competition of the excess androgen with the inhibtor for a common point on, or associated with, these pyridines.

Furthermore, evidence was obtained that related the effects of androgens on pyridine nucleotide coenzymes to the coupled pathway hypothesis of Samuels, Harding and Mann (1962) which may serve as an explanation for the accumulation of fructose and citrate in semen.

ACKNOWLEDGMENTS

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INACTIVATION OF SECRETIN AND CHOLECYSTOKININ CONCENTRATES BY BODY FLUIDS AND ENZYMES

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ABSTRACT.—Secretin concentrates underwent inactivation on incubation at 37°C. with dog, rat and horse sera, canine plasma protein fractions, splenic and hepatic extracts and amniotic fluid as well as with crystalline pepsin but were minimally affected by human urine. The method of isobollic dosages was applied in the determination of secretin activity after various periods of incubation, the ratio of amounts of extract-saline solution to the body fluid mixture, each giving the same pancreatic response in dogs, being a measure of the residual hormonal activity. Some of the cruder products (SI) at 1.0 mg/ml canine serum, displayed inhibition prior to inactivation and the inhibitory phase was diminished or absent with decreasing hormonal concentrations per unit volume of serum. Destruction of secretin activity occurred from the start with SI at 1.0 mg/ml rat serum as was also the case with more highly fractionated types treated with canine serum at the same level but an inhibitory phase was observed with the latter concentrates incubated with horse serum. Although cholecystokinin metabolism paralleled that of secretin in several runs, generally, the rate of inactivation was greater for secretin.

The presence of an enzyme, secretinase, in blood and tissues has been postulated as the causative factor in the *in vitro* inactivation of secretin and in the rapid metabolism of the hormone following intravenous administration (Greengard *et al.*, 1941a). Proteolysis was ruled out on the basis of the relative absence of proteolytic activity in blood serum and in the finding at that time that the hormonal concentrates were not affected by crystalline pepsin and trypsin. Secretinase activity was also demonstrated in urine (Greengard *et al.*, 1941b). Doubilet (1946) regarded the enzyme as similar to choline esterase on the basis of its inhibition by certain drugs and vitamin K. Inactivation of secretin activity by citrated blood plasma in phosphate buffer was likewise observed by Rogers (1951) as was the case with crystalline pepsin and chloroform-activated globulin or serum protease preparation; the cleavage of amino acids did not occur. In this conjunction, the study of Agren and Hammersten (1937) is of interest. Aminipolypeptidase caused the liberation of ten amino acids from a secretin product without any loss in activity. An enzymatic mechanism has also been postulated for the inactivation of cholecystokinin (CCK) by blood serum (Greengard *et al.*, 1941c).

In this study, the method of isobollic dosages advanced by Gershbein *et al.* (1949) was employed for an effective quantitation of the metabolic action of body fluids, tissue extracts and enzymes on secretin and

CCK concentrates. In contrast to the rather qualitative results deduced to date, the present methods allowed for good followup over protracted periods of time and the data could be applied to kinetic evaluations, among others.

Although very potent concentrates are available and in fact, a pure peptide with secretin activity has been synthesized (Bodanszky *et al.*, 1967), the current study with various types of preparations was motivated more by metabolic considerations in relation to 'non-active' moieties associated with or occurring concurrently with secretin in the upper intestinal extracts and which may greatly influence the mode of inactivation as suggested in this report.

Materials and Methods

The secretin concentrates were isolated from the salted acid upper intestinal product of the pig, the A-precipitate, by extraction with 80% ethanol, removal of the alcohol from the filtered solution under vacuum, isoelectric precipitation and heating. The activity was precipitated with trichloroacetic acid to yield the SI, the latter then being converted to the VII-type by treatment with amines in aqueous acetone solution (Gershbein, 1968; Gershbein and Denton, 1967). The acetone-dried A-precipitate (DA) was also used in conjunction with ethanol up to 95% and the activity (ADA) removed by the addition of excess acetone. The ADA was enriched by various treatment schemes. including a picric acid step, the latter giving rise to the P-concentrate (Gershbein and Krup, 1952). In a few runs, clinical samples of secretin (Vitrum) prepared by the methods of the Jorpes' group were also included. The concentrates screened in this study ranged from 0.5 to 300 u/mg for secretin and the CCK extracts contained up to 50 u/mg.

Blood was collected from dogs by cannulation of the jugular and pooled and from horses at the abattoir; rat blood was removed by cardiac puncture. Canine amniotic fluid and blood were pooled during the last quarter of pregnancy. Extracts of dog liver and spleen were prepared by homogenization of the tissue with saline followed by centrifugation of the blend. Spinal fluid was secured from dogs by spinal puncture. Crystalline pepsin (Armour) and a lyophilized concentrate of hog kidney diamine oxidase (DO; Winthrop) containing 1.0 u/mg were employed in the metabolic experiments.

For the evaluation of secretin inactivation by the method of isobollic dosages, a solution of the secretin concentrate in serum or tissue extract, generally at 1.0 mg/ml was incubated at 37°C. together with the control containing an equivalent of the same product in saline. At definite intervals, an aliquot of the serum mixture was removed and injected intravenously into the pentobarbitalized dog with the main pancreatic duct cannulated and the submaximal response (drops of juice) over a period of 10 min ascertained; blood pressure was followed by carotid cannulation. When basal conditions obtained, the animal was standardized against the incubated saline solution. The ratio of dosages of control to serum-treated concentrate, each giving the same response was determined and represented the secretin activity remaining in the incubated mixture. Especially for longer observation periods, asepsis was applied to the bleeding, removal of the serum, preparation of the solutions and to the incubation. The comparisons were carried out against the same saline standard throughout and with a similar mode of handling and where smaller volumes were instituted for rechecking or amplification of values, the corresponding saline control was included at that time. At least two dogs were used in each of the runs.

The metabolism of CCK was also followed by an analogous method and often in the same dog with the pancreatic preparation. The cystic duct was ligated and a metal trocar connected to a glass tube as manometer, secured in the dome of the gall bladder (Gershbein *et al.*, 1949). The corresponding ratio of dosages yielding an equivalent submaximal contractility of the viscus in mm was determined and served as a measure of the residual CCK activity in the incubated mixture.

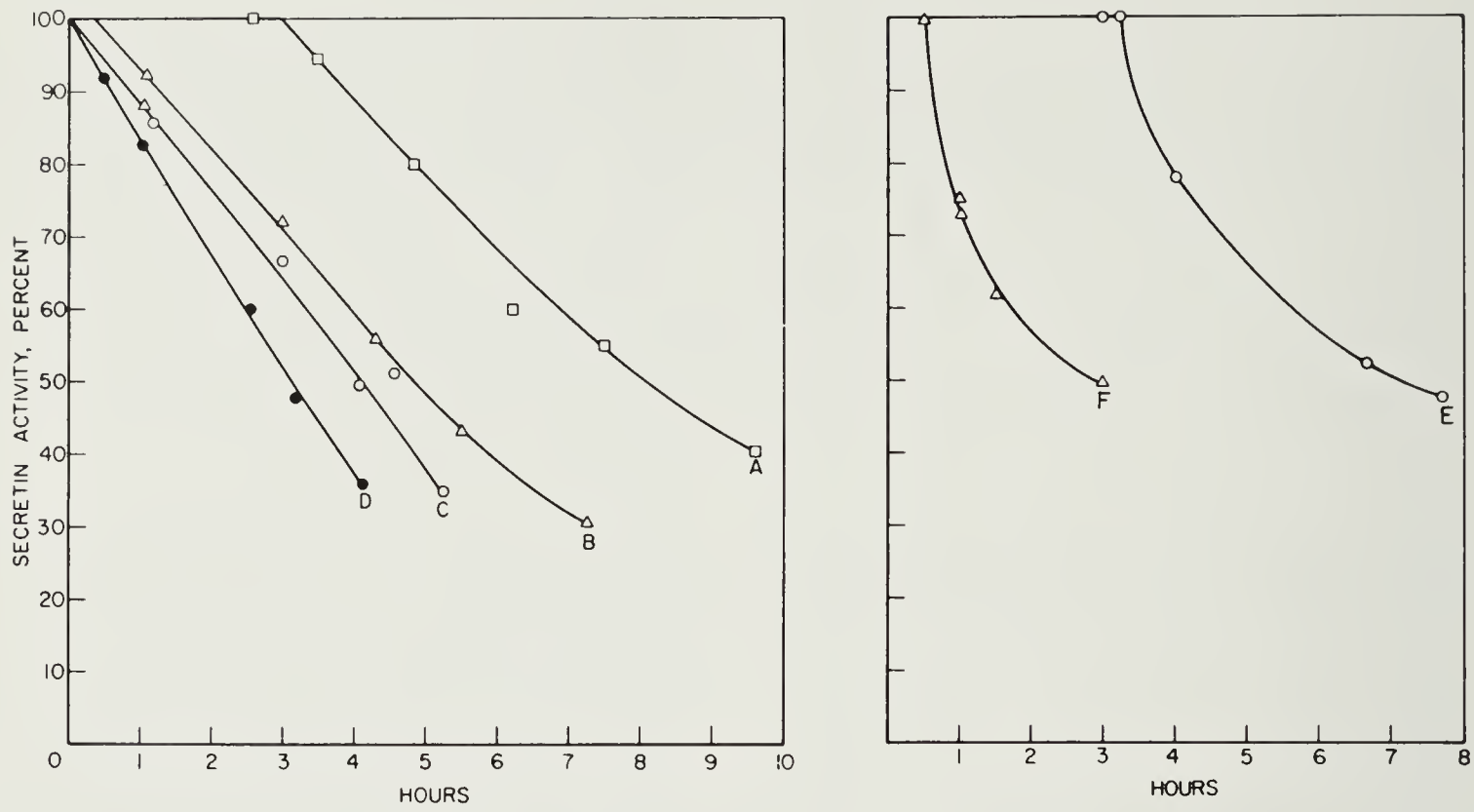

FIGURE 1. Curves depicting the loss of secretin activity on incubation of SI-6 (0.65 u/mg) solutions containing 1.0 mg concentrate in A, 1.0, B, 5 and C, 10 ml of canine serum pool 91 as compared to curve D, SI-6 at 1.0 mg/ml rat serum. E and F were obtained with SI-3 (1.0 u/mg) and SI-4 (4.0 u/mg), respectively, at 1.0 mg/ml canine serum pool 71.

RESULTS

Inactivation of Secretin by Canine Blood Serum

An inhibitory period of about 3 hr was generally noted on incubation of SI concentrates, assaying 0.5-12.5 u/mg, with canine serum at about 1.0 mg/ml before inactivation of the hormone proceeded as exemplified by SI-6 (0.65 u/mg) in Figure 1. The inhibitory period was considerably decreased with a concentration of 1.0 mg per 5 ml of pool 91 (0.13 u/ml) as compared to the 1.0 mg/ml level and essentially absent with the higher dilution by serum (0.065 u/ml); the extent of secretin metabolism increased with diminishing levels of hormonal extract per unit volume of serum (curves A, B and C). SI-3 and SI-4, assaying for 1.0 and 4.0 u/mg, respectively, were also incubated with pool 91 at 1.0 mg/ml and whereas SI-3 simulated the above, SI-4 underwent greater inactivation after a very short inhibitory period (curves E and F). The latter was completely absent on treatment of SI-4 with a second pool but at 1.0 mg/4 ml or 1.0 u/ml (curve C; Figure 2).

The incubation of VII-type concentrates at 1.0 mg/ml serum caused rapid destruction of the hormone without any inhibitory phase even with secretin assaying down to 0.20 u/mg. Typical data are presented in Table 1 for two of the products and illustrated in curves A and B (Figure 2) for a further pool (No. 58) in conjunction with VII-L and VII-R, assaying for 2.0 and 12.5 u/mg, respectively, at 1.0 mg/ml. VII-D (10 u/mg) in addition to the picric acid-fractionated types derived from ADA, P-2 (24 u/mg) and P-10 (50 u/mg) at 1.0 mg/ml and the last product also tested at a level of 1.0 mg per 3 ml of serum pool 82, yield-

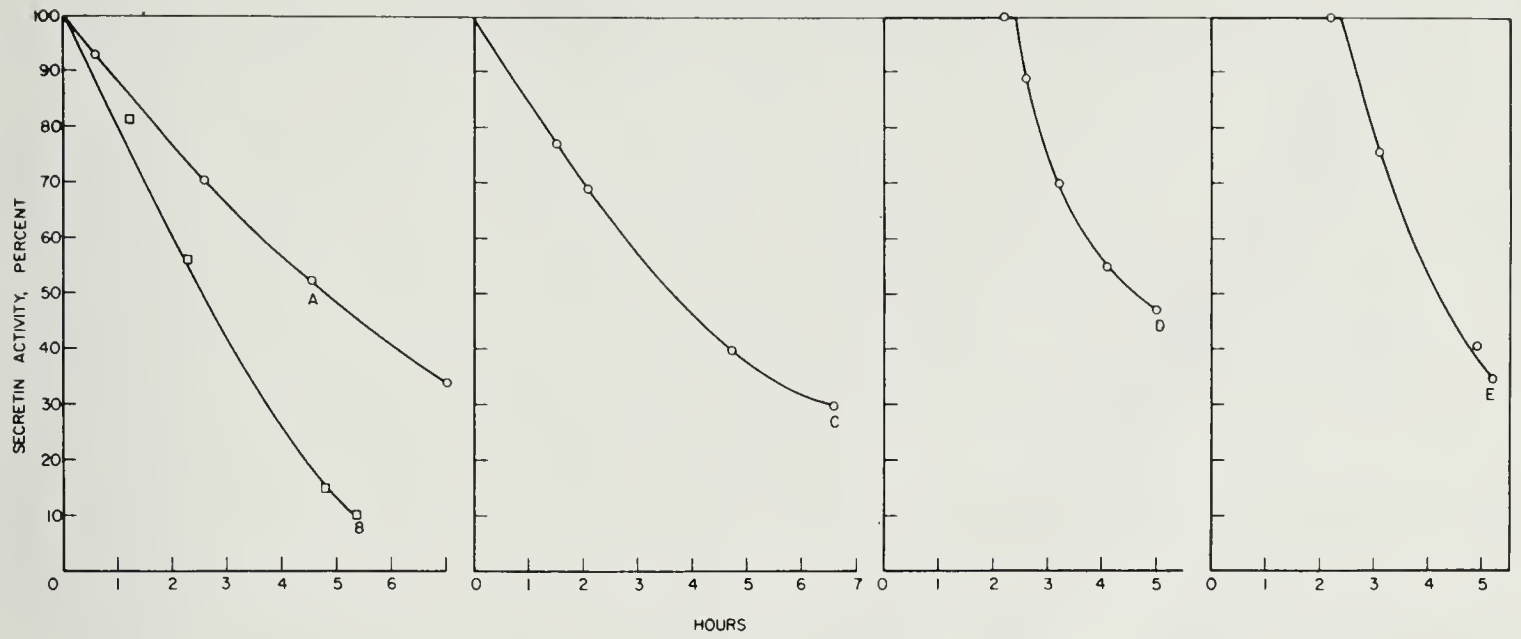

FIGURE 2. Incubation of concentrates with canine body fluids and showing secretin losses with A, VII-L (2.0 u/mg) and B, VII-R (12.5 u/mg) with serum pool 58; C, SI-4 + serum pool 94; D, SI-6 + late pregnancy serum and E, SI-6 with amniotic fluid. In each case, the concentrate has 1.0 mg/ml except for SI-4 at 0.25 mg or 1.0 u/ml.

TABLE 1.—Inactivation of Secretin Concentrates by Canine Serum Pool 70 (1.0 mg/ml).

Incubation Time, min	% Residual Secretin Activity
VII-K (Assay: 0.67 u/mg)	
60	86
153	72
270	52
390	39
VII-R (Assay: 12.5 u/mg)	
60	81
139	56
285	15
323	10

ed the results as advanced graphically in Figure 3. In this regard, the findings with the Vitrum samples, employed up to 300 u/ml, simulated the latter curve, over 80% of the activity being destroyed in 2 hr.

Molecular oxygen was not required for the inactivation of secretin. Thus, the activity remaining 4.25 hr after incubation of SI-4 in serum (1.0 mg/ml) in sealed evacuated tubes was 34% as comparted to 37% after 4.2 hr with the same system in the presence of air.

In all instances, the injection of the incubated sera as well as the tissue extracts and enzyme solutions without hormone at the maximal volumes employed in the respective runs or even higher did not alter the basal pancreatic secretion.

Canine Pregnancy Serum and Amniotic Fluid

The course of inactivation of SI-6 at 1.0 mg/ml of serum pooled from two dogs in late pregnancy as well as of amniotic fluid from such animals, is shown in curves D and E of Figure 2. The inhibitory periods were shorter in either case as compared to sera from nongravid dogs. The incubation of P-2 with the above serum at the same level, led to a loss of 15% of the activity by the end of the first hour and far under 50% after 2.3 hr, whereas, a similar mixture prepared from non-pregnant female serum showed a loss of 27% at the latter period.

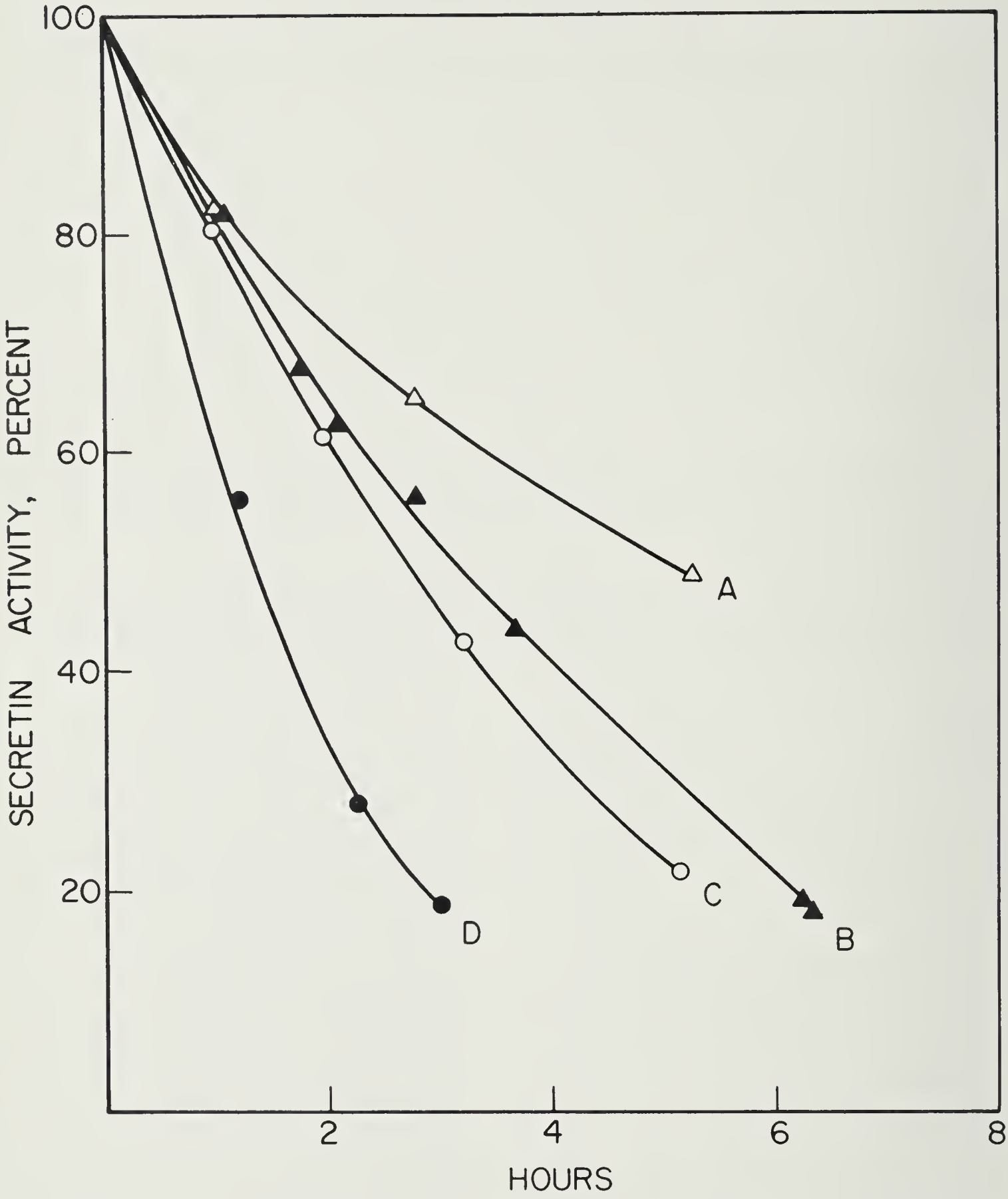

FIGURE 3. Loss of secretin activity with time on incubation of concentrates with canine serum pool 82. A, VII-D (10 u/mg), B, P-2 (24 u/mg) and C, P-10 (50 u/mg) at 1.0 mg/ml serum and D, the last concentrate at 1.0 mg/3 ml serum.

Rat Serum

With serum pooled from rats of either sex, the extent of inactivation of secretin in SI-6 at 1.0 mg/ml exceeded that of the same concentrate or of SI-4 incubated at 1.0 mg/ml and 1.0 mg/4 ml, respectively and no inhibition was observed (curve D; Figure 1).

Horse Serum

Secretin concentrates were treated with serum pooled from mares, stal-

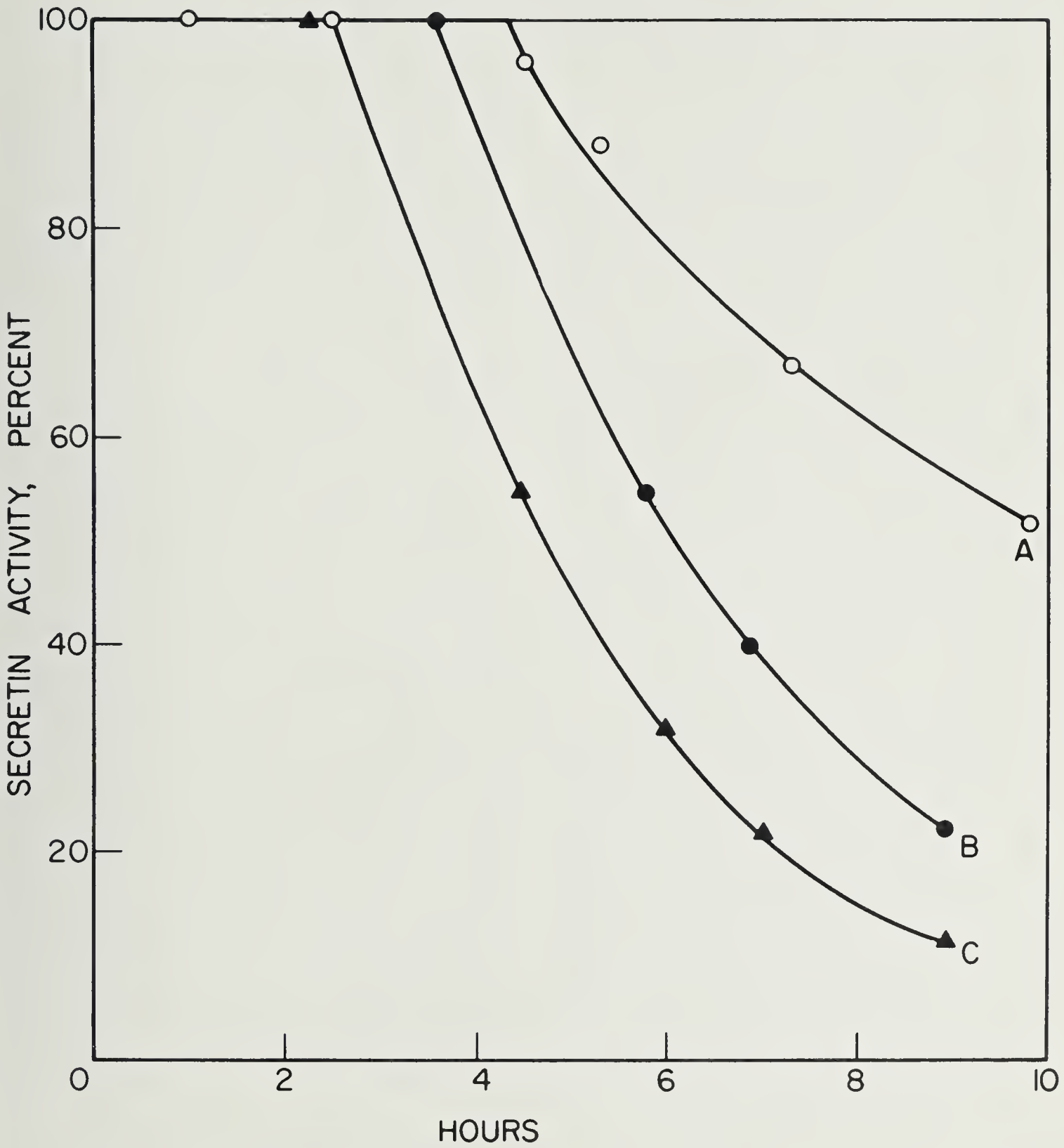

FIGURE. 4. Treatment of concentrates at 1.0 mg/ml horse serum. A, VII-L and pool 10; B and C, VII-R incubated with pools 10 and 12, respectively.

lions and geldings, representative data of which are presented graphically for VII-L and VII-R in Figure 4. The first extract was incubated with a pool from three mares and the second one, with the latter as well as an additional pool, all at 1.0 mg/ml serum. A similar metabolic pattern was obtained with VII-R treated with a pool from four geldings, the secretin activity remaining after 7.3 hr being 67% as was also noted with the mare serum.

Canine Tissue Extracts

On incubation of SI-15 (2.0 u/mg) at 1.0 mg/ml of a 1:80 liver-saline extract, 88% of the hormone was inactivated in 53 min. Under comparable conditions, treatment with 1:300 spleen extract for 50 min caused a loss of 79% and with dilu-

tion of the spleen extract to 1:1,000, 25% was destroyed in 70 min.

Urine and Spinal Fluid

Little metabolism of secretin occurred on incubation of various concentrates with a pool of normal human urine at 1.0 mg/ml and the injection of the mixtures or of the urine alone, generally affected the condition of the assay animal and its standardization. A possible loss of 20% in activity was noted after incubation of 1.0 mg VII-N (9.1 u/mg) in 5 ml of urine for 8.5 hr. Canine spinal fluid of normal or low protein content was essentially without effect on several potent extracts following incubation for 2 hr.

Canine Blood Plasma Fractions

Preliminary screening was carried out with canine plasma fractions I, II, III, IV-1, IV-4 and V isolated by 'method 6' of Cohn *et al.* (1946) and each reconstituted to the respective original level in plasma. Incubation of SI and VII extracts at 1.0 mg/ml of protein solution for 6 hr, led to hormonal losses which were somewhat lower or comparable to those employing the starting fluid or the various serum pools.

Enzymes

Pepsin. - ADA-4 (6.6 u/mg) and P-12 (16 u/mg), each at 1.0 mg per ml of a solution of 1.0 mg% crystalline pepsin in saline, yielded the data depicted graphically in Figure 5 over a period of 1 hr. A similar solution but containing VII-M (9.5 u/mg) underwent somewhat lower metabolism or simulated the behavior of P-12, the residual activities at 35 and 65 min being 42 and 18%, respectively.

DO. - Of several concentrates screened, SI-11 (6.0 u/mg) at 1.0 mg/ml of a saline solution containing 1.0 u DO and P-12, introduced in mixtures with DO at 0.10, 0.50, 1.0 and 2.5 u/ml, underwent losses in hormonal activity, a short inhibitory period occurring with the 0.10 u-level (Figure 6).

Anomalous Behavior Among Animals in Assay of Secretin Concentrates

By the usual *in vivo* assay procedure for secretin (Gershbein *et al.*, 1949), SI, VII and ADA, among other types, yielded virtually identical potency ratios or values from one dog to another as compared to SI or VII standard extracts. However, with about 10-15% of the standardized animals, the assay values were almost half of the expected one with various concentrates, especially those in which a picric acid treatment step was instituted in their fractionation. This, P-4 assayed for 9.0 u/mg in each of 11 dogs but repeated assay produced a value of 4.5 in one animal and 5.0 u/mg, in yet another.

CCK Inactivation

CCK which was quite prominent in SI and VII, in fact, the latter displaying a 1:1 secretin-CCK correspondence in relation to SI (Gershbein, 1968), was followed during the incubation with serum, tissue extracts and enzymes. With many of the SI and VII concentrates, the metabolism of CCK paralleled that of secretin, notably with the higher serum dilutions but usually the rate of inactivation of CCK appeared to be much slower. With SI-4 at 1.0

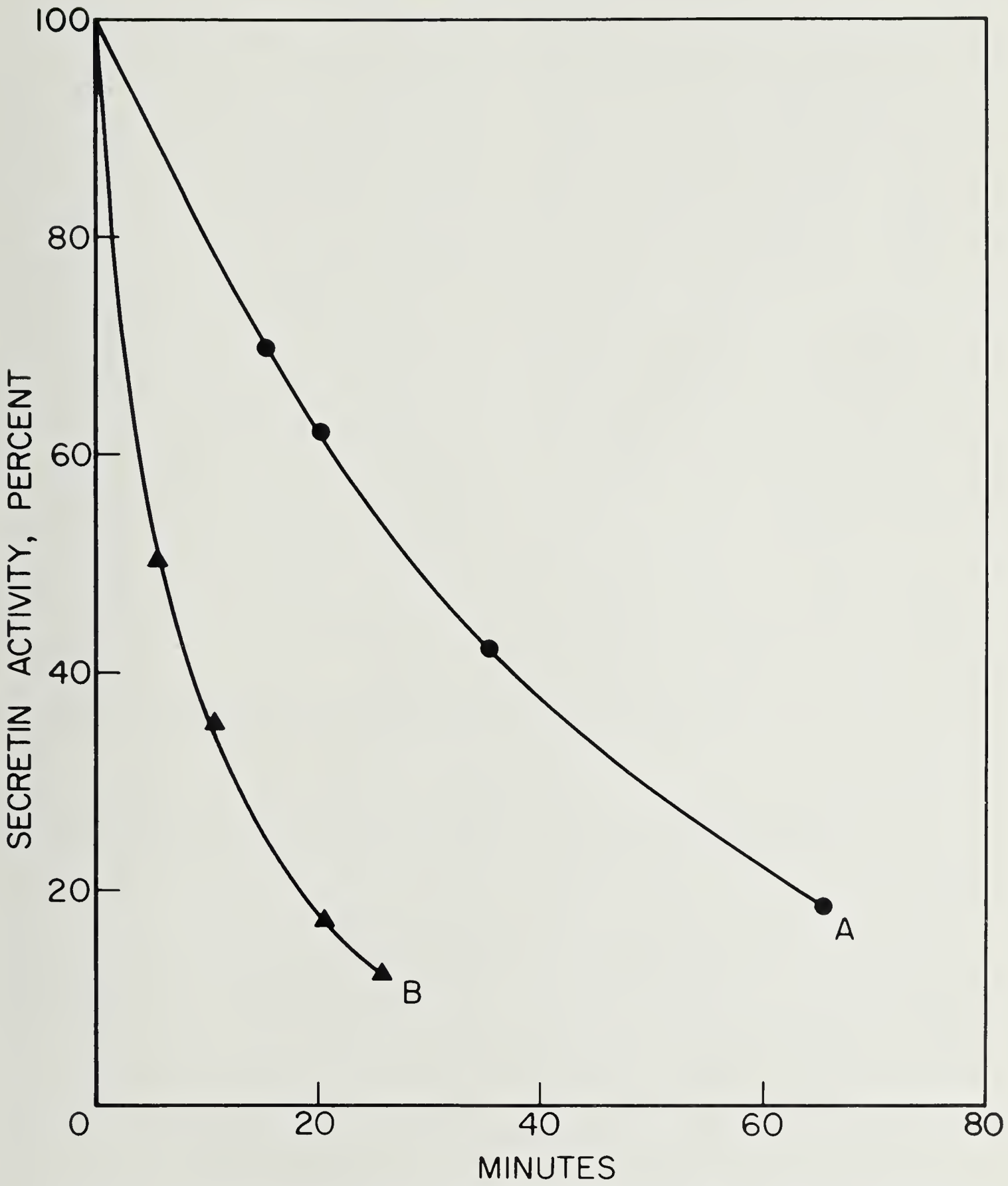

FIGURE 5. A, P-12 (16 u/mg) and B, ADA-4 (6.6 u/mg), each incubated at 1.0 mg/ml of a 1.0 mg% solution of crystalline pepsin in saline.

mg per 5 ml of canine serum at 2.5 hr, both secretin and CCK underwent losses of 31% and a correlation also obtained with SI-6 treated with rat or canine serum. However, incubation of VII-N with dog serum at 1.0 mg/ml for 6.5 hr led to losses of 71 and 29% in secretin and CCK, respectively; in the case of VII-L at a comparable level with horse sera, the residual secretin and CCK activities were 48% each at 9.8 hr. The extent of inactivation of secretin by the DO concentrate also appeared to exceed that of CCK. With SI-11 at 1 mg/u DO, the secretin and CCK

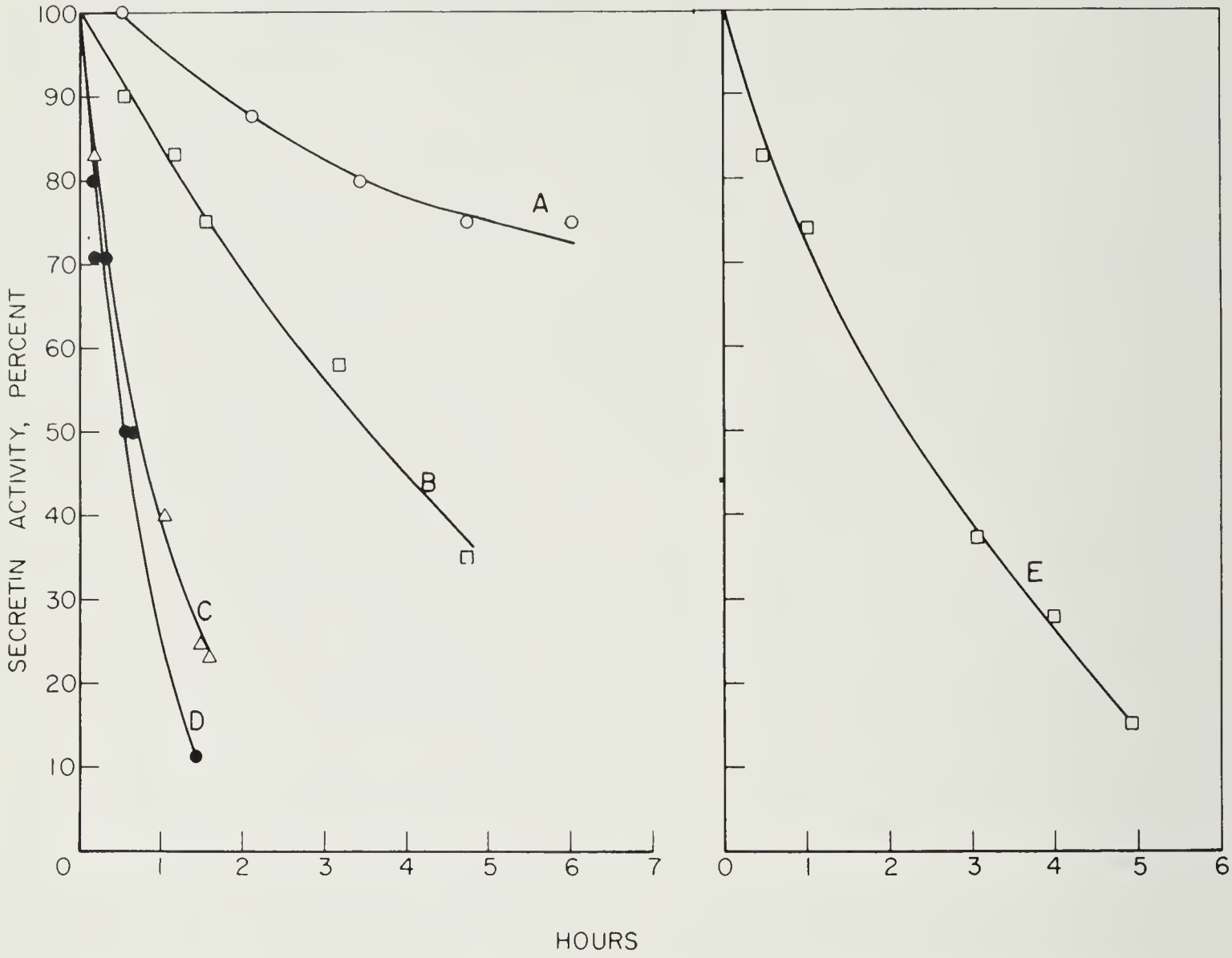

FIGURE 6. Metabolism of P-12 at 1.0 mg/ml of saline -DO solutions containing A, 0.10, B, 0.50, C, 1.0 and D, 2.5 u per ml; E, SI-11 (6.0 u/mg) incubated at 1.0 mg/u DO.

contents at 4.9 hr were 20 and 65% respectively. In another series employing 0.10 u DO/ml, SI-15 displayed activities of 25 and 75% for secretin and CCK, respectively, 18 hr after the start of incubation.

DISCUSSION

The inactivation of secretin in various concentrates has been followed over protracted periods of incubation at 37°C. with serum pooled from such species as the dog, horse and rat. With SI products assaying 0.5-12.5 u/mg, a rather constant finding was the initial inhibitory period occurring at levels of 1.0 mg/ml canine serum and which decreased in duration when the dosage per unit serum volume was diminished (Figure 1): Although the inhibitory phase at 1.0 mg/ml was shorter with SI-4 (4.0 u/mg) and no longer present at 1.0 mg/4 ml as compared to SI-6 (0.65 u/mg), this did not appear to stem solely from the elevated initial unitage. With VII-type extracts as well as ADA and its derived concentrates, destruction of secretin occurred from the start and progressed at rates commensurate with the initial activity or unit concentration per ml of canine serum.

The inhibitory interval noted in the inactivation of SI may originate from preliminary binding with serum protein components prior to pos-

sible enzymatic attack of the transitional product or complex. In yet another approach, it is likely that an enzymatic inhibitor may occur in the preparations, as reflected in the initial phases, considering that VII of equivalent or far lower activity than the SI screened, exhibited no inhibition with canine serum. Also, a definite species difference was evident as regards the observed SI metabolism, under comparable conditions, rat serum causing secretin destruction from the start. However, inhibitors might occur in the serum as well as in the concentrates or in both, considering that VII displayed long inhibitory periods, up to 4-6 hr, on incubation with horse serum (Figure 4).

In the consideration of the effect as due to an enzyme, its concentration in serum may vary in extremes between horse and rat, canine serum ranging intermediately. The postulation of a unique or discrete enzyme, secretinase, rather than of a protease, by Greengard *et al.* (1941a), might not be justified on the basis of their experiments, according to which serum was purported to lack proteolytic acitvity and crystalline pepsin and trypsin, to be without influence on the hormone. Such findings are in marked contradistinction to those of Rogers (1951), among others. Also, early studies had demonstrated that gastric juice rapidly destroys secretin (Carlson *et al.*, 1916). The inactivation by trypsin was attributed by the Greengard group to the alkalinity of the incubated test mixture as such. The enzymatic approach was amplified in the present investigation. Crystalline pepsin was found to destroy secretin activity and in fact, the make-up of the concentrate influenced the action. In this regard, the metabolism of the picrate-purified products appeared to be anomalous in relation to the assay of secretin, 2-3 out of 20 dogs yielding ratios in terms of SI or VII standards of about half of the anticipated value. Accordingly, the type and arrangement of 'inert' components have a great effect on the metabolic properties of the extracts. Such considerations also entered into the efficacy of various schemes in the fractionation of secretin concentrates discussed earlier (Gershbein and Krup, 1952).

The inactivation of secretin by blood serum proceeds in the absence of oxygen and likewise occurs with the plasma protein fractions obtained by the Cohn methods. Canine pregnancy serum appeared to inactivate the hormone more rapidly than nongravid female serum and amniotic fluid was also effective. In contrast to the latter, the destruction of hormonal activity by human urine was minimal.

Although loss of secretin activity was noted with moderate to high levels of the DO product, further purification will be required to pinpoint the metabolic efficacy of the enzyme and to rule out others, notably cathepsins, before significance can be attached to these findings. Additional experiments are necessary to ascertain whether the rapid metabolism of secretin extracts engendered by dilute hepatic and splenic homogenates is on the same or on a different basis compared to blood serum. Although CCK destruction correlated with that of secretin in several instances employing concentrates con-

taining both principles, the extent of inactivation of CCK was generally of a lower order.

The mechanism of secretin metabolism by body fluids and tissue extracts is still open and the role of inhibitors residing in the hormonal concentrates, in the fluid or possibly, in both, is yet to be delineated. With the ease and efficacy of the assay procedures in the screening of incubated mixtures, such secretin studies may provide an attractive model in following the metabolism of proteinaceous hormones of other endocrine systems. The isolation of a pure heptacosapeptide, notwithstanding, the *in vivo* metabolism of secretin must involve preliminary binding with subsequent breakdown of higher molecular weight complexes, discrete units recognized by the body in hormonal metabolic pathways. An investigation of the binding of the heptacosapeptide with blood proteins and tissue extracts would be very timely.

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INEXPENSIVE CHEMICAL INSTRUMENTATION: STUDENT COULOTITRATORS

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ABSTRACT.—Instructions are presented for construction and operation of three models of inexpensive constant-current devices for coulometric titrations, one of which is completely self-contained. These simple and sturdy devices yield excellent analytical results comparable to those obtained from considerably more expensive commercial instruments.

Historically, either indirect chemical means (silver or gas coulometers) or electonic constant-current devices have been utilized to determine the number of coulombs used in coulometric titrations (Reilley, 1954). While the chemical devices are not particularly expensive to fabricate (although a silver coulometer can be expensive to operate), they do require a substantial amount of analytical weighing, equipment manipulation, and numerical calculations. Commercial electronic constant-current devices, on the other hand, are convenient and simple to operate, but are relatively expensive —so much so that they are generally unattainable to the average high school or small college.

The objectives described in previous articles of this series (Van Atta, Linnenbom, and Coleman, 1967; Coleman and Van Atta, 1967, 1968) have been applied to the design, construction, and testing of constant-current coulotitrator devices. As in previous communications, the instruments are identified by the name "Valco" for inventory purposes in the authors' laboratory. Presented are (1) a coulometric accessory (Valco II), (2) a compact unit employing an external timer (Valco III), and (3) a completely self-contained dual-range coulotitrator (Valco SC).

MATERIALS

Lists of electrical and other components required for construction may be obtained, on request, from the senior author. Construction tools required include screw driver, appropriate pliers, a small adjustable wrench, 100-watt soldering iron, multicore solder, nibbling tool, drills, and chassis punches.

Equipment required for calibration and testing of the instruments included a Valco self-contained potentiometer and a vacuum tube voltmeter (for checking output of high-voltage power supplies).

A titration cell of the authors' own design, illustrated in Figure 1, was employed. The platinum wire spiral generator electrode and similar isolated auxiliary electrode are fitted in a 100-ml beaker. The isolated electrode consists of a pyrex tube (½-inch O. D.) fitted with a medium porosity glass frit (alternatively, a hard-packed plug of glass wool is suitable). This compart-

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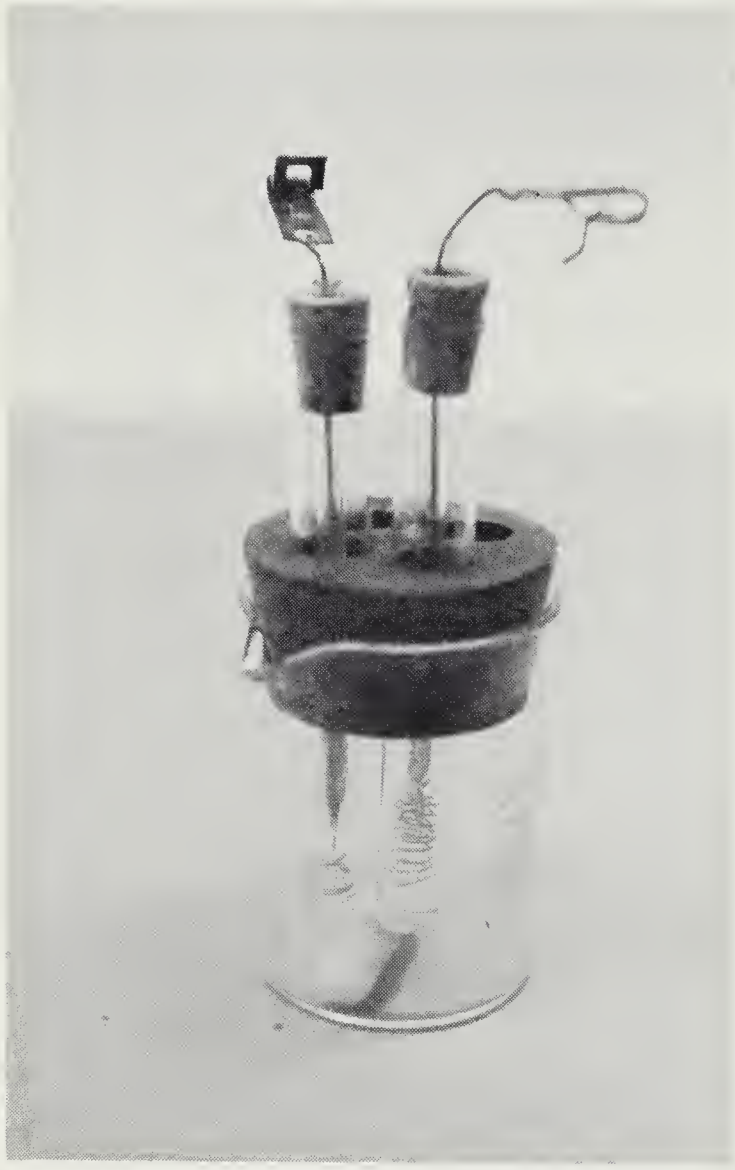

FIGURE 1.—Coulometric Titration Cell.

ment is filled with an electrolyte appropriate to the particular titration being performed.

Chemicals used were all analytical reagent grade. Thorn Smith (Royal Oak, Michigan) analyzed student samples were employed as standard samples for instrument performance tests.

CONSTRUCTION DETAILS

Coulometric Accessory (Valco II).— A very inexpensive device (approximately $10.00) suitable for performing coulometric titrations may be constructed on a breadboard layout. The schematic circuit, adapted from Waser (1964, pp. 284-290) is shown in Figure 2. This device is accurate enough for most routine titrations, simple to construct, and easy to operate, although it lacks the overall convenience of the other devices subsequently described.

The "regulator circuit", shown enclosed in dashed lines in Figure 2, is fundamental to all three instruments described, serving as the mechanism for controlling the generator current. Some external source of B+ voltage is required (preferably 100-200 volts DC, although the unit may be operated on voltages as small as 12 volts DC); Heathkit B+ eliminators or similar devices are quite convenient for this purpose. An external timer, such as a stop watch, is also necessary; an ordinary sweep second-hand watch is satisfactory when titration times in excess of about 10 minutes are employed. Parts layout is not critical in construction of this device, although proper insulation of high voltage connections is essential.

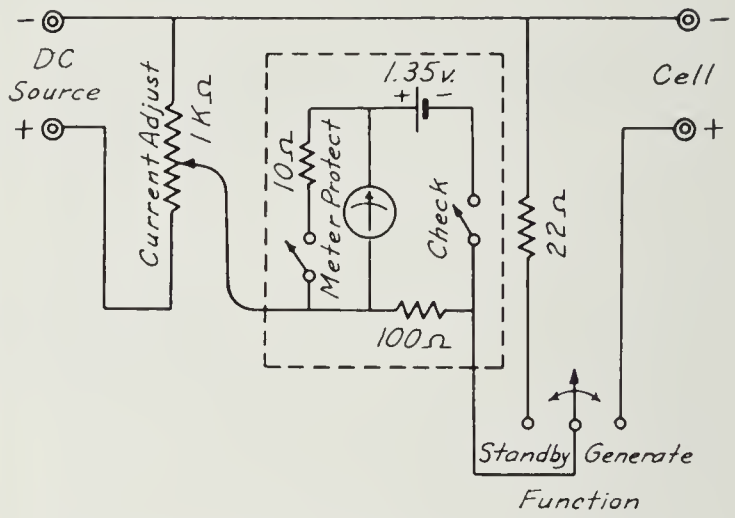

FIGURE 2.—Schematic Wiring Diagram for Coulometric Accessory. Meter: ± 100 μA tuning meter; 100 ohm resistor: ±1%, ½-watt.

The potential of the 1.35-volt mercury battery is accurately determined with a potentiometer such as the Valco model. The external B+ supply is connected with the Function Switch in the "standby" position; the Current Adjust control is then positioned so that meter null is obtained when the Check button is depressed (coarse adjustment is made first with the Meter Protect switch closed) and the titration is initiated by simultaneously starting the timing device and throwing the Function Switch to "generate". The meter is immediately checked for null; meter null is maintained during the course of the titration by appropriate operation of the Current Adjust control as required. The visual endpoint employed depends on the particular chemical system under investigation.

The current supplied to the cell is governed by Ohm's Law:

$$i_{cell} = \frac{E_{battery}}{R_{std}}$$

where $E_{battery}$ is the measured poten-

tial of the mercury battery and R_{std} is the resistance of the precision resistor in the regulator circuit (100.0 ohms). Once the current level and endpoint time measurements are obtained, analytical calculation for the amount of substance reacted (based on Faraday's Laws) is simple and straightforward:

$$\frac{\text{Weight substance reacted}}{\text{Equivalent weight}} = \frac{\text{Current x Time}}{\text{96500 coulombs/equivalent}}$$

The Valco II accessory is an advantageous device for use in high schools or small colleges where instruction in the technique of constant current coulometric titrations is desired, but funds are severely limited.

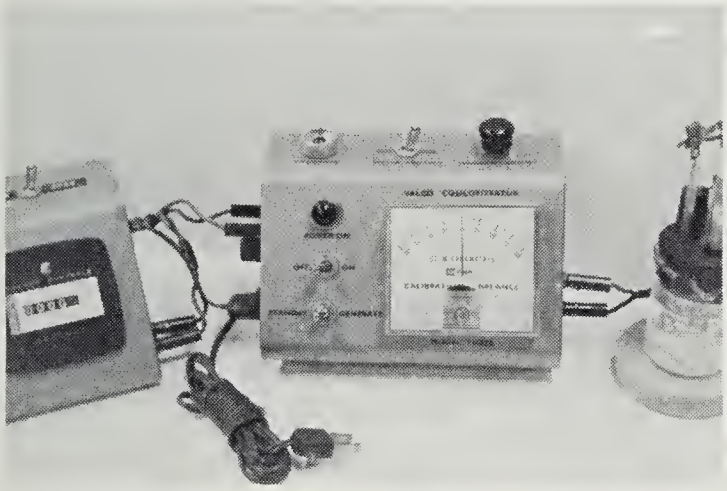

FIGURE 3.—Compact Coulotitrator.

Compact Coulotitrator (Valco III).—The compact coulotitrator (Figure 3) is identical in principle to the accessory model except for the incorporation of an internal power supply and a good quality microammeter, along with provision for direct electrical connection with an electrical timing device and the use of generally higher quality components (total cost under $30.00). The circuit diagram is identical to that for the subsequently described Valco SC, Figure 4, except that a single 100-ohm calibrating resistor and an external timing device are employed. Greater accuracy of current measurement may be obtained through the use of higher quality (and more expensive) precision resistor(s). The internal power supply may be assembled from conventional components available from usual electronics supply houses. It should be noted, however, that instruments incorporating this power supply may show excessive heating of the Current Adjust control if operated continuously for more than about 30 minutes per hour.

The Valco III is operated in the same manner as the accessory model previously described. Although it is somewhat more expensive (under $60.00), it is considerably more convenient to use.

Dual-Range Self-Contained Coulotitrator (Valco SC).—Illustrated in Figure 5 is a dual-range, completely self-contained coulotitrator with integral timer and power supply; the schematic circuit is shown in Figure 4. The operational principle and procedure with the dual-range instrument are the same as those for the other two instruments, except for much greater convenience of manipulation during titrations. Generator currents of approximately 13.5 or 27.0 milliamperes may be realized (exact current depending on the mercury battery output). Other ranges and multiple combinations may be employed, if desired. The timing device is wired into the instrument so that it may be operated independently of the coulotitrator circuit, permitting the external timer" circuit to activate an external circuit or to be used as a simple timing device.

Performance and Application

A wide variety of systems may be employed in constant-current coulometric titrations, involving either anodic or cathodic generation of titrant. An excellent summary of the procedures, fundamental principles, and applications has been presented by Reilley (1954). Two systems were selected for checking performance characteristics of the Valco instruments: (1) Cathodic generation of OH^- from a Na_2SO_4 electrolyte (phenolphthalein endpoint indicator) for the determination of the purity of potassium acid phthalate samples, and (2) anodic generation of I_2 from an $NaHCO_3$-KI electrolyte (starch endpoint indicator) for the determination of the As_2O_3 content of unknown arsenite samples.

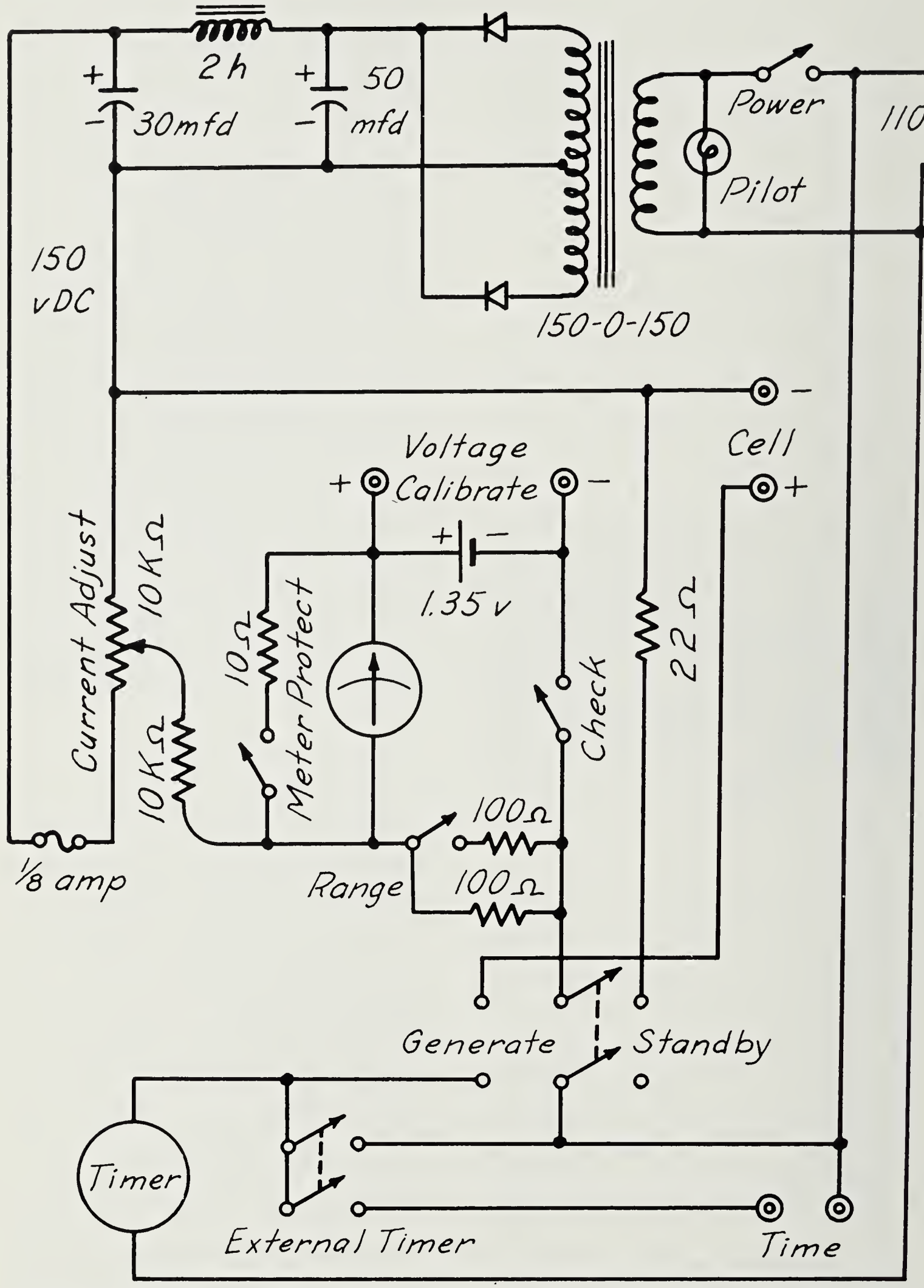

FIGURE 4.—Schematic Wiring Diagram for Self-Contained Coulotitrator. Meter: 100-0-100 μA; 100-ohm resistors: ± 1%, ½-watt or better; timer: Cramer Type 6335 resettable running time meter, 1/10 to 9999.9 seconds.

TABLE 1.—Performance Characteristics of Valco Coulotitrators.

Instrument	Timing Device	% $KHC_8H_4O_4$* Taken	Found	% As_2O_3* Taken	Found
Valco II	Wrist watch, sweep-second hand	50.07	50.20	14.10	14.22
Valco III	Stop watch	34.46	34.52	3.35	3.44
	Coupled electric timer	20.12	20.16	5.70	5.72
		34.46	34.50	14.10	14.15
Valco SC	Integral timer	20.12	20.14	3.35	3.34
		34.46	34.49	5.70	5.68
		50.07	50.15	14.10	14.12

* Results corrected for indicator-electrolyte titration blank.

FIGURE 5.—Dual-Rrange Self Contained Coulotitrator.

The experimental procedure used for the neutralization titrations was reported by Van Lente, Van Atta and Willard (1959); that for the redox titrations has been described by Van Atta (1968, pp. 17-18X). Results of these tests, converted to percentage data, are shown in Table 4. These data, taken by high school students who had never before performed a titration of any kind, illustrate the accuracy which may be obtained for typical titrations by inexperienced students employing this equipment.

ACKNOWLEDGMENTS

The authors acknowledge the support of the National Science Foundation, whose grant GW-595 (1966) supported much of the preliminary experimentation leading to development of the devices described, and of the Cooperative Research in Metal Complex Chemistry Project of the Carbondale Campus of Southern Illinois University for the purchase of electrical components and financial support for D. M. C.

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ACTIVITY AND USE OF HABITAT BY RADIO-TAGGED COTTONTAILS DURING WINTER

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ABSTRACT.—Cottontails were radio-tracked on an orchard during 18 winter nights including one night with 4 inches of wet snow on the ground and one night with blizzardlike conditions. During the latter two nights, rabbits traveled less than during other nights. With wet snow on the ground, they remained in dense woody cover; with blizzardlike conditions, they primarily utilized stands of unmowed weedy forbs and grasses. On 16 nights with moderate weather, individual animals utilized habitat in a manner suggesting cover preferences, but there was little consistency in use of cover types among rabbits. Use of an autumn-planted cover crop of oats as food and a tendency to avoid areas without woody overstory were indicated. Suggestions for improving the methods used in this study are given.

The habits of cottontail rabbits (*Sylvilagus floridanus*) have been difficult to study because of the nocturnal nature and the apparently generalized habitat requirements of this species. The objectives of this study were to measure activity and use of habitat types by cottontails during winter by means of radiotelemetry.

METHODS

Sixteen cottontails, seven males and nine females, were radio-tracked during winter nights on the University of Illinois orchard near Urbana. Observations were made during January 10 through February 10, 1966, and during February 6, through 28, 1967.

Study Area.—The study area was an experimental orchard, approximately ½ mile square, having well-defined blocks of habitat arranged in rectangular patterns (Fig. 1 *A*). The most common woody plants present were apple (*Pyrus Malus*), and pear (*Pyrus communis*), and peach (*Prunus persice*), trees, and grapes (*Vitis* spp.). Common herbaceous plants were bluegrass (*Poa* spp.), timothy (*Phleum* spp.), foxtail grass (*Setaria* spp.), chicory (*Cichorium intybus*), prickly lettuce (*Lactuca scariola*), plantain (*Plantago* spp.), goldenrod (*Solidago* spp.), and pigweed (*Amaranthus* spp.). About half of the area was mowed at least once each summer.

Each block of habitat was classified according to the density of its overstory and to the type of herbaceous ground cover present. The following classes were used:

Ground cover:
1. Mowed vegetation, primarily grass (Fig. 1 *D*)
2. Plowed ground
3. Unmowed grass (Fig. 1 *F*)
4. Unmowed weedy forbs or grasses and forbs (Figs. 1 *C* and *E*)
5. Partly mowed forbs or grasses and forbs (strips mowed among grape arbors—Fig. 1 *B*)

Overstory (woody vegetation over 2 feet tall):
1. None
2. Sparse to medium (crowns not touching—Figs. 1 *D* and *F*)
3. Dense (crowns mostly touching; includes grape arbors—Figs. 1 *B* and *C*)

Trapping and Tracking Procedure.—Movements of seven cottontails were studied during 1966, nine during 1967. They weighed between 710 and 1,390 g

FIGURE 1.—*A*, aerial photograph of the study area taken during the summer of 1966; *B*, grape arbors—dense woody overstory with partly mowed forbs and grasses; *C*, dense woody overstory with unmowed forbs and grasses; *D*, sparse woody overstory with mowed grass; *E*, Foreground—unmowed weedy forbs; *F*, sparse woody overstory with autumn-planted cover crop of oats.

each when captured. Each animal was livetrapped, sexed, eartagged, weighed, fitted with a collar-mounted radio transmitter, and released where captured on the study area. The rabbits were radio-located every 15 minutes between 4 or 5 PM and midnight (CST) during from one to nine nights each (mean = 4.9 nights/rabbit). Tracking was conducted on 18 nights and a total of 72 rabbit-nights of data were obtained.

Transmitters used on the rabbits were designed by Cochran (1967); maximum ranges of the transmitters on cottontails varied between 3/8 and ½ mile.

Movements of the 16 rabbits were monitored using a truck-mounted and a stationary receiver in 1966 and two truck-mounted receivers in 1967. Each receiver had a manually rotated, directional receiving antenna 10-25 feet above the ground. Each antenna was a two-yagi interferometer providing a sharp null between two signal peaks. The receivers were stationed approximately 700 feet apart.

System error (Heezen and Tester, 1967) averaged ± 1.9°. Average distance between 29 positions of test transmitters scattered over the study area and radio-indicated positions of these transmitters was 32 feet. Heezen and Tester (1967) have noted that system error increases in any direction from the optimum location, which is the 90-degree intersection of the bearing lines at the perpendicular bisector of the base line. In the present study, animals were trapped and released near the perpendicular bisector of the base line and receiving equipment was occasionally moved to improve accuracy. As a result, most of the animals' activities occurred in good locations for accurate tracking and the average error in locating rabbits is believed to have been less than 30 feet.

Data Analysis.—Data were analyzed using the University of Illinois IBM 7094 computer. A plotting program similar to one described by Siniff and Tester (1965) was used to draw maps of daily cottontail movements. Activity of each rabbit was measured by computing the average distance between successive radio-indicated locations taken at 15-minute intervals.

Home range limits were determined using all data for each rabbit and drawing lines between the outermost locations recorded, according to the minimum home range method as described by Mohr (1947). Home range area was computed and the area of each habitat type within each home range was measured using an aerial photograph.

A rabbit moving in a random manner within its home range would be expected to use habitat types in proportion to their prevalence within the home range. To evaluate use of habitat by each rabbit, the number of observations within each habitat type was compared with the number of observations expected within the type under conditions of random movement. Chi-square (Steel and Torrie, 1960: 352) was used to evaluate differences. Significant positive chi-square values represent animals using cover types more than expected under conditions of random movement. Significant negative chi-square values represent animals using cover types less than expected. If the rabbits' movements within their home ranges were primarily at random, few significant chi-square values would be expected. Conversely, if the rabbits' movements were primarily nonrandom, many significant chi-square values would be expected.

Since activity of rabbits and their use of habitat appeared to be affected by weather conditions, the data were grouped into (*a*) nights with 0-1 inch of snow on the ground, (*b*) a night with 4 inches of wet snow on the ground, and (*c*) a windy night with blowing and drifting snow. Respectively, these groups included (*a*) 16 nights with a total of 16 rabbits and 65 rabbit-nights; (*b*) 1 night with 2 rabbits; and (*c*) 1 night with 5 rabbits.

Results

Activity.—On nights with 0-1 inch of snow on the ground, the indicated average distance that rabbits moved during 15-minute periods between 4 PM and midnight was 77 feet (Fig. 2). Excluding the first hour which included data on rabbits that appeared to be sitting in daytime forms, the indicated average distance moved per 15 minutes varied between 66 and 95 feet.

Cottontail movements were reduced during the night with 4 inches

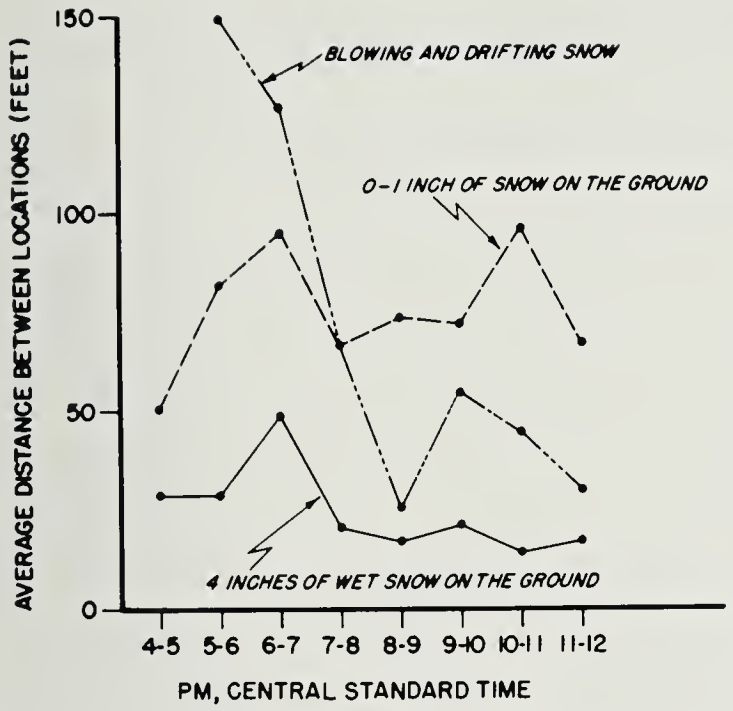
150
100
50
0
AVERAGE DISTANCE BETWEEN LOCATIONS (FEET)
BLOWING AND DRIFTING SNOW
0-1 INCH OF SNOW ON THE GROUND
4 INCHES OF WET SNOW ON THE GROUND
4-5 5-6 6-7 7-8 8-9 9-10 10-11 11-12
PM, CENTRAL STANDARD TIME

FIGURE 2.—Activity of radio-tagged cottontails during hourly periods on winter nights with different weather conditions. Activity is measured by average distance between radio-indicatel locations taken at 15-minute intervals.

of wet snow on the ground; the radio-indicated average distance moved per 15 minutes was 25 feet (Fig. 2). Although 25 feet approximated the average error of the tracking system, it was confirmed that the rabbits were active since some long movements occurred, some consecutive short moves in one direction occurred, and movements could be detected by changes in signal strength.

On the night with blowing and drifting snow, the radio-indicated average distance moved per 15 minutes decreased sharply from 149 feet during 5 to 6 PM to an average of 38 feet during 8 PM to midnight (Fig. 2). The early-evening movement of the rabbits appeared to be from daytime resting cover into habitat offering more shelter, since the blizzardlike conditions began in the late afternoon and increased in intensity during the tracking period.

Habitat Use.—Regardless of weather conditions, movements of the rabbits within their home ranges were not at random. Nonrandom movement, indicated by the numerous significant chi-square values in Tables 1, 2, and 3, suggests that rabbits were preferring some types of cover.

However, on nights with 0-1 inch of snow on the ground there was little consistency in use of habitat types among rabbits (Table 1). Consequently, when data for all animals were combined, average use of most cover types approximated the average prevalence of the types within the rabbits' home ranges (Table 1). The only notable exceptions were a preference for unmowed grasses in the understory and avoidance of areas with no woody overstory (Table 1). Of 16 rabbits, 8 used areas without woody cover slightly less than expected. Five of six cottontails having plots of an autumn-sown cover crop of unmowed, sparsely planted oats (Fig. 1*F*) within their home ranges used these plots more than expected, but the sixth cottontail appeared to avoid the oat plots.

On the average, 63 percent of the area within the home ranges of radio-tagged rabbits provided no understory cover (mowed and plowed ground, Table 1). On nights with 0-1 inch of snow on the ground, the animals showed no consistent tendency to avoid these open areas (as in Fig. 1*D*) and 57 percent of the radio observations indicated rabbits using mowed or plowed ground (Table 1). Even a freezing rain on January 12, 1966, did not result in a change in this pattern of cover use.

On February 1, 1966, with 4 inches of wet snow on the ground, the two rabbits being radio-tracked remained

TABLE 1.—Average Habitat Composition of the Home Ranges of 16 Cottontails and Their Use of Habitat Types during 16 Winter Nights with 0-1 Inch of Snow on the Ground.

Habitat Type	Average Composition of Home Ranges* (percent)	Average Habitat Use† (percent)	Number of Home Ranges Containing Habitat Type	Number of Rabbits Using Habitat Types Either More or Less Than Expected ($P<0.05$) More	Less
Understory					
Mowed grass	54	52	16	2	4
Plowed ground	9	5	13	2	4
Unmowed grass, including oats	3	6	6	5	1
Unmowed forbs and grass	14	17	16	5	2
Forbs and grass, mowed in strips	19	19	16	3	3
Unclassified	3	1	11	1	1
Total	102	100			
Woody overstory (2-20 feet tall)					
None	42	32	16	0	8
Sparse, crowns not touching	16	22	16	6	3
Dense, crowns mostly touching	39	44	15	6	2
Unclassified	3	1	10	0	1
Total	100	99			

* Averages are compiled from the percentages of each habitat type within each home range.

† For each rabbit, the percentages of observations that occurred in each habitat type were computed. These percentages were averaged to obtain figures in this column.

in habitat with dense woody overstory and with either mowed or partly mowed weedy forbs and/or grasses in the understory, (Fig. 1*B*, 1*C*). Chi-square values (Table 2) indicate their preferences for woody cover and their avoidance of areas, within their home ranges, that did not have woody cover.

On February 23, 1967, with blizzardlike conditions developing in late afternoon and persisting through the period of radio-tracking, five rabbits were being monitored. These animals spent most of their time in mixed stands of unmowed weedy forbs and grasses (Fig. 1*E*), especially after 8 PM, as snow drifts became deeper on the west edges of habitat blocks with ground vegeta-

TABLE 2.—Average Habitat Composition of the Home Ranges of two Cottontails and Their Use of Habitat Types during a Night with 4 Inches of Wet Snow on the Ground.

Habitat Type	Average Composition of Home Ranges* (percent)	Average Habitat Use† (percent)	Number of Home Ranges Containing Habitat Type	Number of Rabbits Using Habitat Types Either More or Less Than Expected ($P<0.05$) More	Less
Understory					
Mowed grass	45	0	2	0	2
Plowed ground	17	0	2	0	2
Unmowed grass, including oats	14	0	2	0	1
Unmowed forbs and grass	14	25	2	1	1
Forbs and grass, mowed in strips	9	75	2	2	0
Unclassified	1	0	1	0	0
Total	100	100			
Woody overstory (2-20 feet tall)					
None	45	0	2	0	2
Sparse, crowns not touching	14	0	2	0	0
Dense, crowns mostly touching	41	100	2	2	0
Unclassified	1	0	1	0	0
Total	101	100			

* Averages are compiled from the percentages of each habitat type within each home range.

† For each rabbit, the percentages of observations that occurred in each habitat type were computed. These percentages were averaged to obtain figures in this column.

tion. Most of these habitat blocks did not have woody overstories. Chi-square values (Table 3) indicate the rabbits' avoidance of open areas and their preference for areas with vegetation capable of stopping blowing snow near the ground.

DISCUSSION

On winter nights without deep snow or blizazrd conditions, this technique for studying cover use by cottontails failed to produce much evidence of cover preferences. While individual rabbits appeared to prefer certain cover types and to avoid others, there were few consistent patterns of apparent preference and avoidance among the animals monitored. This situation could have re-

TABLE 3.—Average Habitat Composition of the Home Ranges of five Cottontails and Their Use of Habitat Types during a Windy Night with Blowing and Drifting Snow.

Habitat Type	Average Composition of Home Ranges* (percent)	Average Habitat Use† (percent)	Number of Home Ranges Containing Habitat Type	Number of Rabbits Using Habitat Types Either More or Less Than Expected ($P<0.05$) More	Less
Understory					
Mowed grass	49	11	5	0	4
Plowed ground	9	0	5	0	1
Unmowed forbs and grass	21	61	5	4	0
Forbs and grass, mowed in strips	23	28	5	1	1
Total	102	100			
Woody overstory (2-20 feet tall)					
None	48	66	5	3	1
Sparse, crowns not touching	13	4	5	0	0
Dense, crowns mostly touching	40	30	5	2	2
Total	101	100			

* Averages are compiled from the percentages of each habitat type within each home range.

† For each rabbit, the percentages of observations that occurred in each habitat type were computed. These percentages were averaged to obtain figures in this column.

sulted from one or more of the following possibilities: (1) This species' habitat requirements may be so generalized that in all but severe weather they are fulfilled within many different cover types. (2) Our method for classifying cover types may have been so superficial that we failed to measure the components of the habitat that were important to the animals. (3) Factors other than habitat preference or avoidance may have been more important in determining the rabbits' movements. The last two possibilities have the support of evidence in Table 1. This evidence is the preponderance of significant chi-square values, both positive and negative, indicating that the rabbits were not moving within their home ranges in a random manner but that habitat factors, as measured, were not responsible for their nonrandom movement. Social factors,

perhaps avoidance of other individuals or social groups, may have determined this nonrandom movement. Marsden and Holler (1964:32, Fig. 10) recorded similar nonrandom use of the area within a pen by cottontails from two, apparently territorial, breeding groups.

Data obtained on nights with 0-1 inch of snow on the ground suggested that the rabbits preferred to use a sparse stand of planted oats. The lower portions of these plants remained green throughout the winter and we believe that rabbits were attracted to them as food. Hendrickson (1938), Allen (1939), Hosley (1942), and Tiemeier et al. (1965) have noted that lagomorphs use planted grains as winter food.

During nights without deep snow or blizzard conditions, the rabbits made much use of the extensive areas of mowed grass on the study area. They may have been feeding on these grasses (mostly bluegrass). The rabbits' use of areas without concealing cover near the ground suggests that good habitat for cottontails need not include broad areas of dense herbaceous cover which hinders visibility and travel.

On nights with 0-1 inch of snow on the ground, the rabbits showed a slight tendency to avoid areas without woody cover. This trend was pronounced on the night with 4 inches of snow on the ground, when the animals moved less and remained in areas with dense woody cover. Allen (1939), Haugen (1942), and Johnson and Hendrickson (1958) have cited evidence of increased use of heavy cover by cottontails during winter, especially during periods with deep snow.

During blizzard conditions, the cottontails showed a preference for habitat with unmowed weedy forbs and grasses. This cover offered the most protection from wind and blowing snow near the ground.

Although considerable time and effort were expended in this research, our objective of ascertaining habitat preferences of cottontails during winter was not well met. It is suggested that future research of this type be conducted in such a way as to satisfy as many as possible of the following conditions: (1) All of the animals within the population should be radio-tagged, as can be done with a penned population. (2) Habitat on the study area should be controlled to provide fairly large blocks of homogeneous, well-defined cover types providing distinctly different habitat conditions. (3) Visual observations should supplement radio-tracking to clarify the activities associated with radio-observed movements. (4) The social structure of the observed population should be studied and the movements of the animals should be examined for interactions among individuals.

Acknowledgments

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THE ASEXUAL REPRODUCTION OF *SCHIZOMERIS* AND *ULOTHRIX* (CHLOROPHYTA) IN RESPONSE TO VARIOUS LIGHT INTENSITIES

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ABSTRACT.—The influence of light intensity on the reproduction of a species of *Schizomeris* and the interaction of light intensity and exogenous dextrose on the reproduction of a species of *Ulothrix* were determined. The behavior of *Ulothrix* zoospores with respect to direction of light source and aeration was noted and discussed.

Experiments involving the influence of growth regulating compounds on the growth and reproduction of algae were begun by the first author and his students in 1964. The study reported herein was performed in order to determine optimal light intensities for use in these experiments.

Bold (1942) and Pringsheim (1949) reported that full sunlight was generally inhibitory to the growth of algae. The latter proposed that algae were adapted to growth under relatively low light intensity in nature. Myers (1953) found 400 foot-candles to be optimal for growth of a strain of *Chlorella pyrenoidosa*. Phillips and Myers (1954), working with the same species, reported that 600 foot-candles was optimal when given as short flashes rather than continuously. Myers (1951) reported a light intensity-temperature interaction. Sorokin and Krauss (1958) found that light saturation ranged from 250 to 500-foot candles for five kinds of algae grown at 25°C. They found that light saturation for a high temperature strain of *Chlorella pyrenoidosa* changed from 500 to 1400 foot-candles as the temperature was raised from 25°C to 39°C. Optimal light intensity has been found to vary with the duration and frequency of the light periods (Phillips and Myers, 1954), genetic make-up of the species or strains of algae used (Sorokin and Krauss, 1958), carbon dioxide content of the nutrient medium (Spoehr and Milner, 1949, and Tamiya, 1957), and temperature (Myers, 1951, and Sorokin and Krauss, 1958).

MATERIALS AND METHODS

The culture room was placed on a cycle of 16 hours of illumination and 8 hours of darkness. A light intensity range of 0 to 700 foot-candles was chosen, with 40 watt "cool-white" General Electric fluorescent tubes as the source of illumination, as suggested by Starr (1960). Distance from the light source was used to vary the light intensity, which was measured using a General Electric type-213 light meter. All experiments were performed at 72°F.

A modification of Bristol's solution (Table I) was used as the basic culture

TABLE I.—Composition of modified Bristol's Solution. A liter of final solution contains 10 ml each of stocks 1 through 6 and 1 ml each of stocks 7 through 10.

Stock Solution	Quantity per Liter	Compound
1	25 g	$NaNO_3$
2	2.5 g	$CaCl_2$
3	7.5 g	$MgSO_4.7H_2O$
4	7.5 g	K_2HPO_4
5	17.5 g	KH_2PO_4
6	2.5 g	NaCl
7	50 g	EDTA
	31 g	KOH
8	4.98 g	$FeSO_4.7H_2O$
	1 ml	H_2SO_4, Conc.
9	11.42 g	H_3BO_3
10	8.82 g	$ZnSO_4.7H_2O$
	1.44 g	$MnCl_2.4H_2O$
	1.57 g	$CuSO_4.5H_2O$
	0.71 g	MoO_3
	0.49 g	$Co(NO_3)_2.6H_2O$
	1 ml	H_2SO_4, Conc.

medium. The growth containers were 16 x 150 mm test tubes containing 15 ml of nutrient medium. The tubes of medium were autoclaved at 250°F for 25 minutes. Each experiment was evaluated after seven days under the conditions specified.

A clonal culture of *Schizomeris* sp., having bacterial contaminants, was obtained from H. L. Monoson, then at Kansas University, and utilized in this study. *Schizomeris* was inoculated by delivering 0.1 ml from a 15 ml matured culture by means of a pipette. It was grown under eight light intensities, varying from 0 to 600 foot-candles. Ten replications of the basic design were used in each trial. There were five trials in this experiment, each trial being performed on a different date using fresh inoculum and medium. Reproduction was determined by counting populations of new filaments within the 16 units (1.05 mm^2) of a grid insert placed within the ocular lens system of a microscope. Each test tube was placed on a tilted microscope stage and was evaluated at eight evenly-spaced locations along the tube's circumference in a plane midway between the liquid surface and the bottom of the tube, using a magnification of 100 X.

An axenic culture of *Ulothrix acuminata* Mattox and Bold from the Culture Collection of Algae at Indiana University was also studied. Inoculations were made by transferring one loop (2.5 mm diameter) full of material from an agitated tube of matured filaments. This alga was grown at eight light intensities ranging from 0 to 700 foot-candles. Six replicates of the basic design were included in each trial. There were four trials using the basic culture medium and three with the basic medium plus 1.25 g/1 dextrose.

Glass microscope slides were cut longitudinally and one half-slide was placed in each test tube prior to autoclaving. Reproduction was determined by counting the number of new filaments per 1.05 mm^2 in ten evenly-spaced locations on the side of the glass slide facing the light. The top half-inch of the slide was discounted since it picked up additional filaments during its withdrawal from the medium.

RESULTS

The optimal light intensity for *Schizomeris* within the specified environmental conditions proved to be between 400 and 500 foot-candles (Table II). Maximum populations were found at 400 foot-candles in trials 3 and 4 and at 500 foot-candles in trials 1, 2 and 5. It was determined that *Schizomeris* did not reproduce in the absence of light. New filaments originated from zoospores and the filament count was taken as an approximation of zoospore release.

All trials using *Ulothrix* yielded maximum filament counts at 500 foot-candles with the exception of trial seven in the series having dextrose, which yielded maximum count at 600 foot-candles (Table III). It

TABLE II.—Number of filaments of *Schizomeris* per field (1.05 mm²). Each number represents a mean of eight fields per test tube times ten replications.

	LIGHT INTENSITY (FOOT-CANDLES)							
	0	50	100	200	300	400	500	600
Trial 1	0	2.3	11.5	20.8	30.9	57.5	96.2	55.2
Trial 2	0	2.1	8.6	10.2	21.4	29.6	38.3	26.1
Trial 3	0	0.8	2.2	6.7	4.1	31.7	18.6	11.9
Trial 4	0	1.8	4.8	29.5	33.6	42.3	20.8	16.7
Trial 5	0	2.3	5.4	5.8	13.2	23.0	40.1	23.2
Mean	0	1.8	6.5	14.6	20.6	36.8	42.5	26.6

TABLE III.—Number of filaments of *Ulothrix* per field (1.05 mm²). Each number is the mean of ten fields per slide (test tube) times six replications. The nutrient media were modified Bristol's solution in trials 1 through 4 and modified Bristol's solution with 1.25 g/1 dextrose in trials 5 through 7.

	LIGHT INTENSITY (FOOT-CANDLES)							
	0	100	200	300	400	500	600	700
Trial 1	—	0	4.1	19.6	23.0	27.0	16.8	16.1
Trial 2	—	6.0	18.5	31.1	42.8	94.5	58.6	37.5
Trial 3	—	2.1	8.1	28.8	44.0	75.5	41.5	41.1
Trial 4	2.5	7.3	12.6	14.5	19.5	39.8	24.8	16.3
Mean (1-4)	2.5	5.1	10.8	23.5	32.3	59.2	35.4	27.7
Trial 5	9.5	17.1	28.5	28.8	56.0	72.3	49.6	37.1
Trial 6	9.8	7.1	11.6	21.0	23.5	24.8	24.1	13.0
Trial 7	21.5	13.5	19.5	30.3	33.6	56.3	62.8	32.0
Mean (5-7)	13.6	12.6	19.9	26.7	37.7	51.1	45.5	27.3

was found that *Ulothrix* would reproduce in darkness and that the zoospores would develop into nearly colorless filaments.

Trial three in the inorganic medium series (Table III) was investigated to determine whether phototaxis, which appeared to exist, really occurred. The population counts were consistently higher on the side of the glass slide facing the light than on the side away from the light source (Table IV). The zoospores moved toward the light source prior to cementing themselves to solid objects in their vicinity. This was the case at all light intensities between 100 and 700 foot-candles. Furthermore, the zoospores exhibited a strong aerobic tendency by swimming to the surface of the medium prior to their development. The lat-

TABLE IV.—Number of filaments of *Ulothrix* per field (1.05 mm²). Each number is the mean of ten fields per slide (test tube) times six replications in trial 3 without dextrose.

	LIGHT INTENSITY (FOOT-CANDLES)						
Orientation	100	200	300	400	500	600	700
Facing light	2.1	8.1	28.8	44.0	75.5	41.5	41.1
Opposite light	0.5	7.8	18.8	29.6	38.1	24.3	22.5

ter was concluded from observations rather than from population counts.

DISCUSSION

The data, indicating maximum reproduction of *Schizomeris* and *Ulothrix* at approximately 500 foot-candles, are generally in agreement with the results of Myers (1953), Phillips and Myers (1954), Sorokin and Krauss (1958), and Starr (1960). The literature indicates that saturation light intensity can be shifted by aeration (Spoehr and Milner, 1949, and Tamiya, 1957), temperature (Myers, 1951, and Sorokin and Krauss, 1958), light duration (Phillips and Myers, 1954) and genetic characteristics (Sorokin and Krauss, 1958). It is likely that light quality, inorganic and organic nutrients, pH, and other factors also contribute to determining light saturation.

It has been impossible, thus far, to separate filament size increase from zoospore formation in *Ulothrix,* as both have been stimulated by the same factors. However, the filaments produced under 600-700 foot-candles exhibited shortness, few cells, and an anomalous sort of branch or accessory holdfast production. The dark grown cells were very elongate, with the plastids considerably shorter than the cells' lengths.

The fact that low light intensities are inhibitory to reproduction is of considerable interest as is the reversibility of this inhibition with dextrose. It is not clear, however, whether exogenous dextrose or photosynthate are necessary (1) as respirable materials, (2) as assimilable materials, or (3) as stimuli to the processes of zoosporogenesis and zoospore release. Kulfinski and Soice (1965) found that dextrose was inhibitory to *Ulothrix,* although stimulatory to *Leptosira obovata.*

Kulfinski and Dash (1966) reported dextrose to be stimulatory to growth of *Tribonema aequale.* It is of interest, therefore, that dextrose is stimulatory to *Ulothrix* at certain light intensities and inhibitory at others. Diffusion of gases between the nutrient medium and the atmosphere may be the limiting factor in the utilization of dextrose at 500 foot-candles and perhaps this inhibition can be reversed by aeration of the medium.

Evidence was found that the zoospores of *Ulothrix* are positively phototaxic throughout the range of light intensities employed. The presence of greater numbers of filaments at the surface of the medium indicated that the zoospores are strongly aerotaxic.

Summary

1. Light saturation with respect to zoospore formation and release was found to exist in the vicinity of 500 foot-candles for both *Schizomeris* and *Ulothrix* in modified Bristol's solution.
2. *Ulothrix* reproduced in total darkness. Reproduction in the dark was increased by addition of dextrose.
3. Dextrose was inhibitory to reproduction at saturation light intensity but stimulatory above and below this intensity.
4. The zoospores of *Ulothrix* moved toward the light source, at the light intensities employed, and toward the surface of the liquid nutrient medium, as determined by population distribution after zoospore release.

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REPRODUCTION AND GROWTH OF *CHLOROTYLIUM CATARACTARUM* KUTZ

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ABSTRACT. — *Chlorotylium cataractarum* Kutzing was cultured in soil water and on soil water agar at ± 21-22°C. The alga reproduced asexually by zoospores produced from the short cells of a filament. Each zoospore was pale green, elliptical, and contained two photoreceptive bodies. Released zoospores attached themselves to the substrate by their flagella. The alga exhibited two prostrate vegetative growth patterns. The first growth pattern resulted in either straight or slightly curved filaments; the second was decidedly curved and produced pseudoparenchymatous colonies. Older cultures contained numerous unicells that had very heavy layered cell walls.

Kutzing (1843) characterized *Chlorotylium cataractarum* by its unilateral and lime encrusted filaments. Collins (1909) reported that two types of cells composed the unilaterally branched filaments. One cell type was round, contained a large quantity of chloroplast material, and functioned in asexual reproduction; the other was elongated, hyaline in appearance, and vegetative in function. Mature filaments were composed of 2-3 round reproductive cells that alternated with 3-7 elongated vegetative cells. Each cell had a plate-like or band-like chromatophore that contained a pyrenoid. Asexual reproduction of *C. cataractarum* was by zoospores that possessed 2-16 flagella and originated from the round cells. Printz (1927) reported that red colored cells were also produced by the alga and these were identified as either akinetes or aplanospores. He also stated that bi-flagellated zoospores were produced from the terminal cells and that the palmelloid stage of the alga had a peculiar coloration.

The investigation was undertaken to clarify and elaborate on the reproduction and growth of *C. cataractarum*.

MATERIALS AND METHODS

A culture of *Chlorotylium* was obtained from Silver Lake, Iowa. Francis Drouet, University of Arizona, later identified the species as *C. cataractarum*.

Stock cultures of the alga were maintained in test tubes of either soil water (SW) or 2% soil water agar (SWA). Soil water was prepared by using a one gallon bottle that was filled one-fifth of its total volume with a rich soil, and distilled water was added to the half-gallon mark. The mixture was then autoclaved for 15 minutes at 15 psi. One week later the supernatant liquid was decanted and diluted 1:1 with Chu's (1942) number ten solution. The final liquid was filtered to eliminate all foreign material. Soil water agar was prepared in a similar fashion but 20g Difco agar was added to every liter of amended SW. Both culture media were then sterilized according to Bold (1942).

Algal samples used in this study were

obtained from bottle cultures. Each bottle was capable of holding 8 ounces of liquid and contained a 3″ x 1″ glass microscope slide. The bottles were filled with four ounces of SW and inoculated with two loops of algal material from stock cultures. All of the inoculation procedures were carried out under sterile conditions to avoid contamination of any type. After inoculation the bottles were placed in a north facing window according to Beyerinck (1890) and Bold (1942). No attempt was made to grow the alga under a 12-12 light/dark photoperiod.

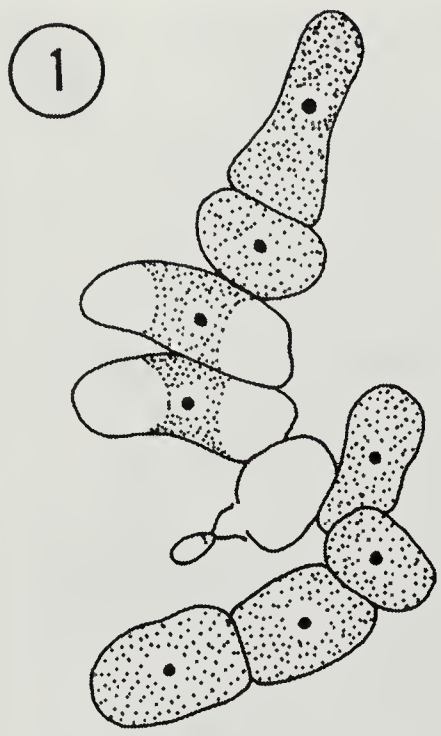
Microscopic observation of algal reproduction and growth was accomplished by the placement of glass slides from the 8 ounce SW bottles onto a continuous-drip apparatus according to Kulfinski, Pappelis, and Monoson (1963). The various growth stages of the alga were recorded as photomicrographs using an Exacta camera.

Results

Zoosporogenesis occurred in *C. cataractarum* 6-8 days after the alga was inoculated into SW bottles. A green ring was noted along the meniscus of the SW solution and suggested that reproduction or growth had taken place. Microscopic observation of the SW slides showed many unicells and small algal colonies on the glass surfaces. The SW slides were then transferred to the continuous-drip apparatus for further observations. Zoospores developed in the round cells of a filament two days after a SW slide was placed on the continuous-drip apparatus. The round cells functioned as zoosporangia and contained 6-8 zoospores. The bi-flagellated zoospores were elliptical, pale green, 9 x 6μ, and contained a cup-shaped chloroplast. An obscure pyrenoid was located in the median portion of the chloroplast. Two photoreceptive bodies were located on either side of the flagellar insertions (Fig. 1). The nucleus and vacuoles of the zoospore were not observed. Zoospores were released from the zoosporangium through a pore in the cell wall and swarmed in a circular fashion for 2-20 minutes. Attachment of these asexual spores to the glass substrate was by their flagella. Once a zoospore was firmly attached to the substrate the flagella were readily reabsorbed by the cell.

Individual cells of *C. cataractarum* were either round, oval, or elongated in shape. The elongated cells often had their chloroplasts centrally located and the end areas of these cells appeared empty (Fig. 2). Short two-, three-, four-, and five-celled filaments were often curved (Fig. 3). A red colored substance was noted along the inner surface of the curved cells. All the algal growth that occurred in SW solutions consisted of one primary filament from which secondary filaments had developed. The secondary filaments were divided into smaller tertiary branches that gave the mature colony a pseudoparenchymatous appearance (Fig. 4).

Zoospores that were inoculated onto SWA plates developed into round, elevated colonies that were composed of short, dark green cells. One-week-old algal colonies on SWA formed unilateral branched filaments that were composed of elongated and hyaline cells. Once again a red colored substance was noted on the inner surface of the curved filaments (Fig. 5). Two-week-old colonies of the alga maintained on SWA plates had disassociated into single and very thick-walled cells (Fig. 6). Microscopic observations were not continued after two weeks and, therefore, it was not possible to determine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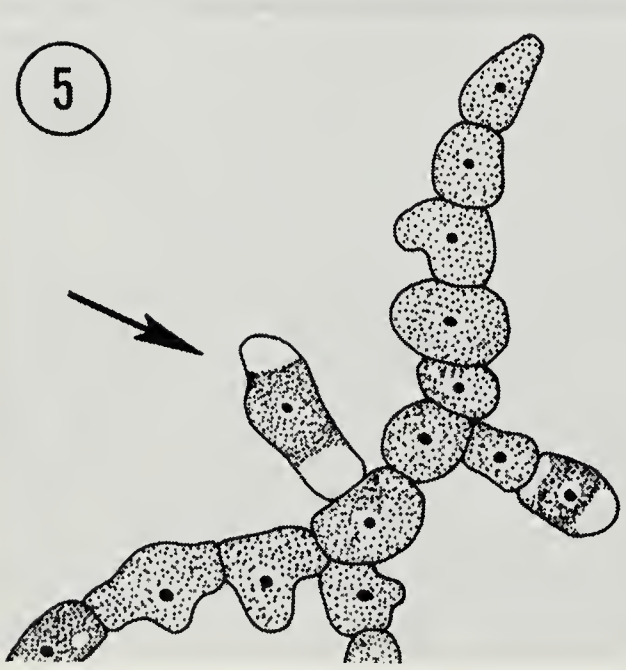
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whether the thick-walled cells were either akinetes or aplanospores.

Discussion

Growth patterns exhibited by *C. cataractarum* were quite different than those reported by other investigators (Kutzing, 1843; Collins, 1909; Printz, 1927; Smith, 1933). The observations of this study have suggested that certain isolates of the alga are heterotrichous. If this were the case the previous reports were of the upright mature filaments and this study dealt with the prostrate anchorage stage from which these upright filaments had originated. At no time during the study were zonal colonies observed as reported by Kutzing (1843).

The development of the algal colony proceeded with the enlargement and division of the original zoospore. Unilateral branching was common in the early stages of development but bilateral branching was more common in older colonies. This type of branch development was again in direct contrast with that described by Collins (1909).

The shape of the cells and filaments were affected by a red colored internal secretion that hitherto had not been reported in the literature. Accumulation of this red substance on one side of the cell resulted in a more rapid growth on the opposite side and curvature of the cell or filament resulted.

Two-week-old colonies contained cells with very thick and layered cell walls. These thick-walled cells readily separated and were possibly the akinetes reported by earlier workers (Collins, 1909; Printz, 1927). If these were in fact akinetes, then any cell of the filament has the potential of becoming an akinete as the culture ages. In the present study, it was impossible to determine whether these thick-walled cells were either akinetes or aplanospores.

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Figures 1-6.—The growth stages of *Chlorotylium cataractarum* Kutz.

1.—Mature filament with zoospore next to a round reproductive cell. X 556.

2.—Young individual algal cells. X 1400.

3.—A curved four-celled filament. Arrows point to the inner cell wall that has a red colored secretion. X 330.

4.—Compact growth pattern due to the early secretion of red colored substance. X 360.

5.—A colony grown on soil water agar. Arrow points to a hyaline cell. X 430.

6.—Two-week-old algal colonies on soil water agar. Note the numerous single cells with heavily layered cell walls. X 1480.

COLEORHIZAL RESPONSES TO CERTAIN PLANT GROWTH REGULATORS IN CULTURES OF EXCISED BARLEY EMBRYOS

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ABSTRACT.—Excised embryos of barley in early stages of development (400-500μ) cultured on synthetic media showed "precocious germination" when cultured on media containing either kinetin or gibberellic acid. In addition to coleoptile elongation, which is a normal germination response in vivo and in vitro, such embryos also developed elongate coleorhizas. The latter response is characteristic of germination in vivo, but not usually of germination in vitro. Coleorhizal elongation was most markedly stimulated by gibberellic acid.

There are two evanescent organs in embryos of grasses which play an important role in germination. The coleoptile, a sheath enclosing the leaf primordia, has received a great deal of attention, not only for the key part it plays in the early life of the seedling but also because of the significant research in auxinology which employs this structure as a reaction system. The coleorhiza, or root sheath, breaks open the covering coats of the caryopsis. In barley and oats, for example, which have tough husks in addition to the seed coats, the coleorhiza forces an opening through layers nearly a millimeter thick. Parenthetically, the coleorhiza is a delicate organ and the forces it employs in its action have never been properly studied.

In studies of excised oat embryos it was observed (Norstog, 1955) that embryos grown in liquid media produced elongated coleorhizas, whereas those grown on solid media did not. In a series of experiments it was shown that embryos grown on a solid substrate in an atmosphere of 2% 0_2 produced similarly elongated coleorhizas. That there was, in addition, an involvement with auxin was demonstrated by experiments in which the addition of indole-3-acetic acid to liquid media resulted in elongations somewhat greater than those occurring in liquid media alone. Aside from those cultures grown in low 0_2, the only agar cultures showing coleorhizal elongations were in two isolated instances on a medium containing 40 mg/1 adenine sulfate.

When excised embryos are cultured at earlier stages of their development they may, depending on cultural conditions, either continue embryonic development or they may grow as small seedlings. The latter development has been termed precocious germination. Recently, in agar

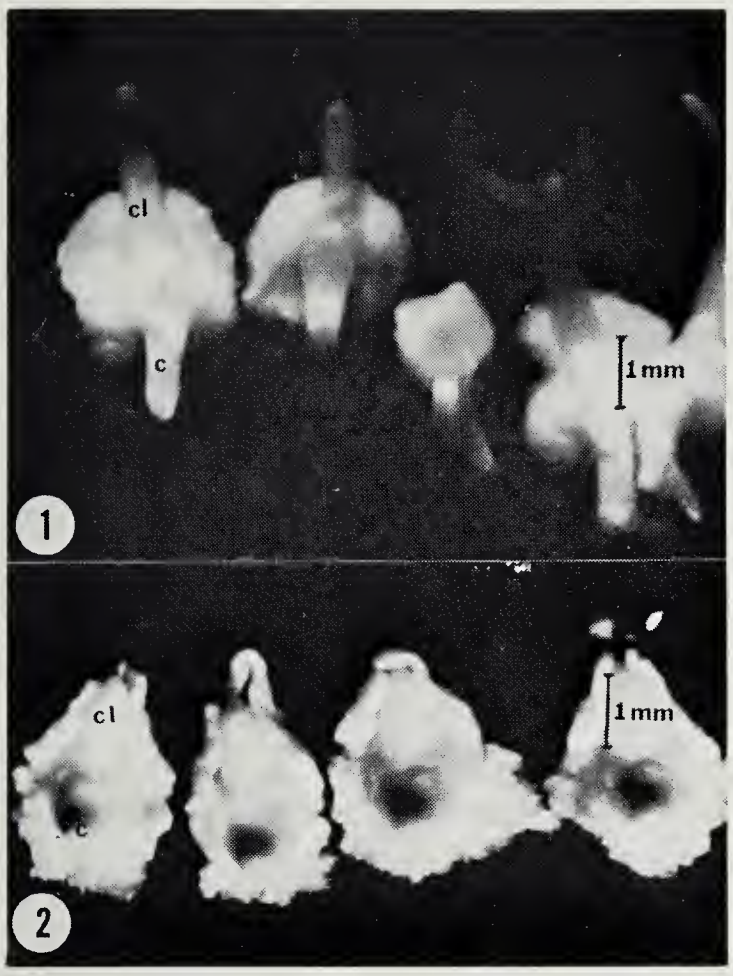

FIGURE 1.—Excised 500μ barley embryos after 10 days' growth on a medium containing 0.1 mg/1 GA_3, at 25° C, in light. Cl-coleoptile, C-coleorhiza.

FIGURE 2.—Excised 500μ barley embryo after 10 days' growth on control medium at 25° C, in light. Cl-coleoptile, C-coleorhiza.

cultures of barley embryos, coleorhizal elongations have been observed in some instances in association with precocious germination. The following brief report deals with those observations.

Materials and Methods

Embryos of barley, *Hordeum vulgare*, var. "Moore", were excised aseptically from still-green caryopses and placed on a synthetic culture medium (Norstog, 1967) which in a ten-day period allowed 500μ embryos to attain a state of development equal to or greater than that of normal full-term embryos (see Fig. 2). These cultures were used as controls against media containing 0.1 mg/1 gibberellic acid (GA_3); 0.1 mg/1 kinetin (6 furfurylaminopurine), (K); and 0.1 mg/1 indole-3-acetic acid (IAA). The latter two substances were used alone and in combination. Cultures were maintained at 25°C in light (20-100 FC) and in dark. The results are summarized in Table 1 and are represented photographically in Figs. 1 and 2. Coleorhizal elongations were observed in all treatments except in those which contained IAA alone. The most consistent results were obtained with GA_3 in light-grown embryos. Coleorhizal elongations occurring in K and K + IAA cultures were somewhat obscured because embryo germination was greatly promoted by these treatments, both in light and in dark, and as germination progressed growing roots ruptured the coleorhiza and its elongation ceased. This was also true of GA_3 cultures in dark, but not so much so in light. It has been observed repeatedly that light tends to inhibit GA_3-induced precocious germination in our barley cultures. Thus dark-cultured embryos with GA_3 displayed ruptured coleorhizas, whereas those grown in light on GA_3 had intact and elongate coleorhizas.

TABLE 1.—Responses of the barley coleorhiza to IAA, K and GA_3.

Treatment	Coleorhizal elongation*: Dark	Light
Control	—	—
GA_3	+	++
K	+	+
IAA	—	—
K + IAA	+	+

* As compared to controls.

Coleorhizal hairs resembling root hairs are a feature of coleorhizal development in some circumstances (Nishimura, 1922; Norstog, 1955). IAA, while not active in the induction of coleorhizal elongation in cultures of barley embryos on solid media, does stimulate coleorhizal hair production in such cultures. There is an interesting morphogenetic dichotomy apparent in this response, in that coleorhizal elongation is usually not accompanied by hair formation, and vice versa.

Discussion

Precocious germination in barley embryo cultures, as in embryo cultures of other species (Rijven, 1952,

1956) represents a marked morphogenetic departure from an embryonic type of development. In the latter, development consists of rapid cell division and differentiation with little if any cell elongation taking place. Precocious germination, on the other hand, may not involve much cell division but is characterized by cellular elongation. Haber and Luippold (1960) have shown in their studies of gamma-irradiated wheat embryos that cell division is not necessarily a corollary of GA_3-induced cell elongation in germination. Differentiation may or may not occur, depending on the nature of the medium and conditions of culture. Although precocious germination may mimic normal germination in many aspects, it occurs without a dormant interval between embryogenesis and the seedling state. Moreover, it may occur before the embryos have reached a full-term state of development. Aside from these differences, there is no reason to believe that precocious germination, as a biochemically-mediated response, differs significantly from normal germination.

The relationship between gibberellin and germination in cereals has been explored by a number of investigators. Gibberellins are involved in the germination response in barley (see for example Paleg, 1960; Yomo and Inumina, 1966; MacLeod and Palmer, 1967; Naylor and Simpson, 1961).

Since in grass embryos germination involves coleorhizal elongation, a case may be made for involvement of gibberellin in the response of this organ in vivo as in vitro.

It is thought that growth in plants is an expression of balanced actions of growth regulators such as cytokinins, gibberellins, and auxins (van Overbeek, 1966). The promotion of coleorhizal elongation in cultures on K and K + IAA media, as well as by GA_3, suggests that interactions between growth substances may affect coleorhizal development. However, biochemical evidence of such relationships has not been obtained as yet.

The observation (Norstog, 1955) that adenine sulfate stimulated coleorhizal elongation in cultured oat embryos is perhaps evidence of cytokinin activity. Doerschug and Miller, 1967, observe that adenine sulfate is a less effective substitute for kinetin in bud induction in *Lactuca* explants. One also may postulate a similarity of action by adenine and kinetin in coleorhizal elongation, with adenine giving a weaker response.

The role of the grass coleorhiza in germination is fundamental. Although it now appears clear that several growth regulators are involved in this action, the interaction of these substances with factors of the physical environment such as light and 0_2 tension is not well understood, and it is apparent that further study of these effects is required.

Acknowledgments

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VEGETATION SURVEY OF FLOODPLAIN FORESTS IN EAST-CENTRAL ILLINOIS

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ABSTRACT.—Woody and herbaceous vegetation surveys were made of six areas (13.75 acres) located in the Embarrass River Floodplain in east-central Illinois. A total of twelve species were found with *Acer saccharinum*, *Populus deltoides*, *Acer negundo*, and *Salix nigra* being the most important. Reproduction in the areas indicate that *Acer negundo* and *Acer saccharinum* will continue to be of major importance. However, there was very little *Populus deltoides* reproduction and *Salix nigra* was only able to reproduce in wet areas near the river's edge. Observations indicate that these floodplain forests are becoming drier since *Celtis occidentalis*, *Ulmus rubra*, *Ulmus americana*, and *Fraxinus americana* are beginning to invade these areas.

The Embarrass River, which has its headwaters in Champaign County, flows south through east-central Illinois for 160 miles and finally enters the Wabash River south of Lawrenceville, Illinois. The river drains most of the fertile farm land in this area and usually floods at least once each year in the latter part of May or early June. The flooded conditions usually last no longer than five days. During the latter part of the summer, the river often becomes so low that a person can jump the small stream in many places.

The Embarrass River was formed as a result of the Wisconsin Glacier which moved into Illinois about 12,000 years ago from the northeast and extended southward and westward to a position along a line from Paris through Charleston, Shelbyville, Decatur, and Peoria. The glacial drift, deposited at this position of the ice front, formed a series of ridges called the Shelbyville Moraine that is built up to an elevation of 700 to 750 feet above sea level (Leighton, Eckblaw, and Horberg, 1948). The proposed dam site of the Lincoln Reservoir is located near the southern edge of this moraine, and the reservoir would occupy the channel cut by the Embarrass River through this moraine and the ground moraine to the north.

The topography of the Shelbyville Moraine in the vicinity of the proposed reservoir is extremely rugged. Numerous small streams enter the steep-walled valley cut by the Embarrass River. Most of these streams are less than two miles from headwater to mouth and flow rapidly down extreme gradients (50-70 feet per mile). Four major tributaries

(Indian Creek, Kickapoo Creek, Whetstone Creek, and Polecat Creek) enter the Embarrass River in the Shelbyville Moraine. These streams are found between the major east-west ridges of the moraine and each has numerous small valleys leading into them. No large or undisturbed floodplain forests are found along these tributaries. Except for near the Embarrass River, these smaller valleys are too narrow and their gradients too steep for the prolonged flooding necessary for the development of floodplain forests.

At one time, the lowland areas along the Embarrass River were covered with floodplain forests. Presently, most of this area has been cleared for agricultural purposes. The few forest areas that remain have been subjected to some cutting and grazing. Since most of this forest type will be inundated by the proposed Lincoln Reservoir, this study was undertaken to determine the forest composition before it was destroyed.

Location of Woodlots Investigated

In this survey the floodplain, referred to as "first bottoms" by Putnam (1951), was considered to be that area of the bottomland which is associated with the present river channel and which normally floods when the river overflows. In each area, a hillside or a terrace formed by an earlier channel served as the limit of the floodplain. In most areas, a natural levee was located just back from the river's edge. Except for small depressions or occasionally an old channel or levee, the areas were relatively flat, seldom varying more than five feet in elevation. All areas were approximately fifteen feet above the present river bed.

Only those areas considered large enough for study and representing relatively mature woodlots were used in this survey. The areas ranged from 0.93 to 3.55 acres, with a total of 13.75 acres surveyed. All six areas surveyed are located along the Embarras River, 4-12 miles south of Charleston, Illinois, and all will be flooded if the proposed Lincoln Reservoir is built.

Method of Study

Each of the six areas studied was divided into 25-meter square quadrats (0.154 acre or 0.0625 hectare) and the number, size, and species of trees were recorded for each. The diameter of all trees with a d.b.h. of more than four inches was recorded to the nearest 1/10 inch.

Dead-standing trees were also measured and identified when possible. Dead-down trees were not recorded as they could have easily been carried into the areas by the frequent floods of the Embarrass River. The Importance Value Index (IVI) was then calculated from the actual data to provide a better basis of comparison of the various species and areas. The IVI, as used here, follows the procedure outlined by McIntosh (1957), in which the IVI is the sum of the

relative density

$$\frac{\text{number of individuals of a species}}{\text{number of individuals of all species}} \times 100,$$

relative dominance

$$\frac{\text{basal area of a species}}{\text{basal area of all species}} \times 100,$$

and the relative frequence

$$\frac{\text{number of plots of occurrence of a species}}{\text{total plots of occurrence of all species}} \times 100.$$

Due to the low frequency of saplings, seedlings, and shrubs their abundance and occurrence in the floodplain forests studied was determined by making a total count in the plots used to study the overstory. Also, the topography and overstory where they were growing was recorded.

All herbaceous species found in the areas studied were identified and their abundance recorded. Collections and observations were made in May, July, and September. The abundance of each species was determined by a modification of the procedure outlined by Acocks (1953). A mean distance was determined after a series of measurements was made between individuals of the same species and the abundance was determined by using the following scale:

Average Distance between Plants	*Abundance Category*
3 in. - 1 ft.	abundant
1 ft. - 3 ft.	common
3 ft. - 12 ft.	frequent
12 ft. - 50 ft.	occasional
50 ft.	rare

Voucher specimens of all species have been deposited in the Eastern Illinois University Herbarium. The taxonomic nomenclature used in this paper follows that of Jones (1963).

Results and Discussion

Twelve species of trees were found in the six floodplain forests studied. Of these, *Acer saccharinum, Populus deltoides,* and *Acer negundo* were extremely important and in most instances had an IVI above 50. *Salix nigra, Ulmus rubra, Platanus occidentalis,* and *Fraxinus americana* were also relatively important in these forests with their IVI's sometimes exceeding 20 but rarely 40. These seven species with their relative values, number of individuals per acre, and basal area per acre in diameter classes for the six areas studied are included in Tables 1 through 6. The remaining species (*Aesculus glabra, Morus rubra, Celtis occidentalis, Gleditsia triacanthos,* and *Juglans nigra*) were rarely encountered and had extremely low IVI's. This latter group of species comprises the "others" in the tables.

Of the arborescent species found in the floodplain forests, *Acer saccharinum* has the highest IVI in all areas studied except area five. It was found in nearly every quadrat examined and in most areas has the highest relative frequency and relative density. This species is well represented in all diameter classes, but is sometimes exceeded by *Populus deltoides* in the larger classes. In area 5 (Table 5), *Acer negundo* exceeds *Acer saccharinum* in the number of individuals per acre in the 4-6 inch diameter class. A few saplings and seedlings of *Acer saccharinum* were found in the floodplain forests. Seedlings were very common at the edge of the forests near the river and some saplings were observed on the higher parts of the floodplain and in small openings in the forest. The number of small diameter trees, as well as the presence of saplings, indicates this species will continue to be an important stand component of the floodplain forests.

Populus deltoides ranks second in IVI in most of the areas studied, mainly because of its high relative dominance. It ranks well below *Acer saccharinum* in relative density, but was well scattered throughout the forests, as indicated by its high relative frequency. This species ranks first in IVI only in area 5 (Table 5). The largest trees found in the survey were *Populus deltoides,* and this species usually had the most individuals and the highest basal

TABLE 1.—Number of Trees and Basal Area Per Acre by Diameter Classes and Relative Values for Area One Located Eight Miles South of Charleston, Coles County, Illinois (NW¼, SW¼, Section 23, T11N, R9E). This area includes 3.55 acres with a variation in elevation of 3.5 feet.

Species	Number of Trees and Basal Area Per Acre by Diameter Class										Relative Values			
	4-6		7-12		13-24		25+		Total					
	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	Rel. Freq.	Rel. Den.	Rel. Dom.	I.V.I.
Acer saccharinum	70.4	11.7	76.1	37.6	23.1	34.8	0.6	2.4	170.2	86.5	26.1	62.4	50.7	139.2
Populus deltoides	6.2	1.1	22.0	12.1	22.0	38.5	3.4	14.5	53.6	66.2	23.8	19.6	38.8	82.2
Acer negundo	12.1	1.9	7.0	2.5	1.7	1.9	.....	.....	20.8	6.3	22.7	7.6	4.3	34.6
Salix nigra	15.2	2.3	3.7	1.6	2.0	2.5	.....	.....	20.9	6.4	9.0	7.6	3.7	20.3
Ulmus rubra	2.5	0.3	0.3	0.2	.....	.....	.....	.....	2.8	0.5	6.8	1.0	0.3	8.1
Platanus occidentalis	0.3	0.1	0.3	0.2	0.8	2.5	.....	.....	1.4	2.8	4.5	0.5	1.6	6.6
Fraxinus americana	2.2	0.4	0.3	0.1	.....	.....	.....	.....	2.5	0.5	4.5	0.9	0.2	5.6
Others	0.6	0.1	.....	.....	.....	.....	.....	.....	0.6	0.1	2.6	0.4	0.4	3.4
Totals	109.5	17.9	109.7	54.3	49.6	80.2	4.0	16.9	272.8	169.3	100.0	100.0	100.0	300.0

TABLE 2.—Number of Trees and Basal Area Per Acre by Diameter Classes and Relative Values for Area Two Located Ten Miles South of Charleston, Cumberland County, Illinois (SW¼, SE¼, Section 27, T11N, R9E). This areas includes 2.32 acres with a variation in elevation of 7.5 feet.

Species	Number of Trees and Basal Area Per Acre by Diameter Class										Relative Values			
	4-6		7-12		13-24		25+		Total					
	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	Rel. Freq.	Rel. Den.	Rel. Dom.	I.V.I.
Acer saccharinum	55.2	9.2	94.8	48.0	21.6	27.2	.....	.....	171.6	84.4	32.6	76.0	60.7	169.3
Populus deltoides	2.6	0.4	7.3	4.4	21.6	37.9	.....	.....	31.5	42.7	26.0	13.9	30.7	70.6
Acer negundo	6.0	1.0	5.6	2.6	1.3	1.8	.....	.....	12.9	5.4	19.5	5.7	3.9	29.1
Salix nigra	0.4	.....	4.7	3.2	2.6	2.6	.....	.....	7.7	5.8	13.0	3.4	4.1	20.5
Ulmus rubra	0.4	0.1	0.4	0.2	.....	.....	.....	.....	0.8	0.3	4.3	0.3	0.1	4.7
Platanus occidentalis	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fraxinus americana	.....	.....	0.4	0.2	.....	.....	.....	.....	0.4	0.2	2.1	0.1	0.1	2.3
Others	0.4	.....	0.4	0.2	.....	.....	.....	.....	0.8	0.2	2.5	0.6	0.4	3.5
Totals	65.0	10.7	113.6	58.8	47.1	69.5	.....	.....	225.7	139.0	100.0	100.0	100.0	300.0

TABLE 3.—Number of Trees and Basal Area Per Acre by Diameter Classes and Relative Values for Area Three Located Seven Miles South of Charleston, Coles County, Illinois (NE¼, NE¼, Section 23, T11N, R9E). This area includes 2.32 acres with a variation in elevation of 5.0 feet.

Species	Number of Trees and Basal Area Per Acre by Diameter Class										Relative Values			
	4-6		7-12		13-24		25+		Total					
	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	Rel. Freq.	Rel. Den.	Rel. Dom.	I.V.I.
Acer saccharinum	45.7	7.2	51.7	26.6	29.7	43.5	.....	.....	127.1	77.3	28.3	56.5	49.4	134.2
Populus deltoides	.....	.....	3.4	2.0	21.1	37.0	1.7	8.0	26.2	47.0	18.8	11.6	30.0	60.4
Acer negundo	17.7	3.0	34.9	17.9	3.4	3.9	.....	.....	56.0	24.8	24.5	24.9	15.8	66.2
Salix nigra	0.9	0.2	1.7	0.9	3.0	4.3	.....	.....	5.6	5.4	7.5	2.4	3.3	13.2
Ulmus rubra	7.3	1.1	0.9	0.3	.....	.....	.....	.....	8.2	1.4	15.0	3.6	0.8	19.4
Platanus occidentalis	.....	.....	0.4	0.2	.....	.....	.....	.....	0.4	0.2	1.8	0.1	0.1	2.0
Fraxinus americana	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Others	0.4	.....	0.9	0.4	.....	.....	.....	.....	1.3	0.4	4.1	0.9	0.6	5.6
Totals	72.0	11.5	93.9	48.3	57.2	88.7	1.7	8.0	224.8	156.5	100.0	100.0	100.0	300.0

TABLE 4.—Number of Trees and Basal Area Per Acre by Diameter Classes and Relative Values for Area Four Located Seven Miles South of Charleston, Coles County, Illinois (NW¼, NE¼, Section 23, T11N, R9E). This area includes 3.09 acres with a variation in elevation of 5.0 feet.

Species	Number of Trees and Basal Area Per Acre by Diameter Class										Relative Values			
	4-6		7-12		13-24		25+		Total					
	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	Rel. Freq.	Rel. Den.	Rel. Dom.	I.V.I.
Acer saccharinum	58.9	9.8	53.4	26.4	9.4	12.1	.....	.....	121.7	48.3	28.1	50.0	37.6	115.7
Populus deltoides	6.2	1.2	22.6	13.0	27.2	45.4	.....	.....	56.0	59.6	25.3	23.0	46.3	94.6
Acer negundo	20.1	2.9	5.8	2.8	0.6	0.9	.....	.....	26.5	6.6	25.3	10.9	5.1	41.3
Salix nigra	20.1	3.0	13.9	7.5	2.6	3.2	.....	.....	36.6	13.7	14.0	15.1	10.6	39.7
Ulmus rubra	1.3	0.2	0.3	0.1	.....	.....	.....	.....	1.6	0.3	4.2	0.6	0.2	5.0
Platanus occidentalis	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fraxinus americana	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Others	0.6	0.1	.....	.....	.....	.....	.....	.....	0.6	0.1	3.1	0.4	0.2	3.7
Totals	107.2	17.2	96.0	49.8	39.8	61.6	.....	.....	243.0	128.6	100.0	100.0	100.0	300.0

TABLE 5.—Number of Trees and Basal Area Per Acre by Diameter Class and Relative Values for Area Five, Located Six Miles South of Charleston, Coles County, Illinois (NW¼, SW¼, Section 13, T11N, R9E). This area incluudes 1.54 acres with a variation in elevation of 5.6 feet.

Species	Number of Trees and Basal Area Per Acre by Diameter Class										Relative Values			
	4-6		7-12		13-24		25+		Total					
	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	Rel. Freq.	Rel. Den.	Rel. Dom.	I.V.I.
Acer saccharinum	9.1	1.5	26.0	13.1	11.7	17.4	3.2	16.6	50.0	48.6	14.8	20.0	28.1	62.9
Populus deltoides	3.9	0.8	39.0	22.4	29.9	45.4	3.2	17.1	76.0	85.7	21.2	30.3	49.6	101.1
Acer negundo	76.6	11.8	18.8	7.0	3.2	4.6	.....	.....	98.6	23.4	21.2	39.4	13.5	74.1
Salix nigra	5.8	0.7	0.6	0.2	.....	.....	.....	.....	6.4	0.9	6.3	2.5	0.5	9.3
Ulmus rubra	3.2	0.5	2.0	0.8	0.6	0.8	0.6	2.8	6.4	4.9	8.5	2.5	2.8	13.8
Platanus occidentalis	0.6	0.1	0.6	0.2	1.3	3.6	.....	.....	2.5	3.9	8.5	1.0	2.2	11.7
Fraxinus americana	0.6	0.1	1.3	0.4	.....	.....	.....	.....	1.9	0.5	2.1	0.7	0.2	3.0
Others	3.9	0.8	1.3	0.7	2.6	3.2	.....	.....	7.8	4.7	17.4	3.6	3.1	24.1
Totals	103.7	16.3	89.6	44.8	49.3	75.0	7.0	36.5	249.6	172.6	100.0	100.0	100.0	300.0

TABLE 6.—Number of Trees and Basal Area Per Acre by Diameter Classes and Relative Values for Area Six Located Six Miles South of Charleston, Coles County, Illinois (NE¼, SE¼, Setcion 14, T11N, R9E). This area includes 0.93 acres with a variation in elevation of 3.8 feet.

Species	Number of Trees and Basal Area Per Acre by Diameter Class										Relative Values			
	4-6		7-12		13-24		25+		Total					
	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	Rel. Freq.	Rel. Den.	Rel. Dom.	I.V.I.
Acer saccharinum	49.5	7.5	62.4	30.6	37.6	60.2	2.2	9.5	151.7	107.8	28.5	55.0	51.1	134.6
Populus deltoides	3.2	0.7	20.4	11.3	28.0	38.6	4.3	17.0	55.9	67.6	14.2	20.3	32.1	66.6
Acer negundo	22.6	3.8	18.3	7.9	8.6	9.8	.....	.....	49.5	21.5	28.5	17.9	10.1	56.5
Salix nigra	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Ulmus rubra	7.5	1.2	3.2	1.2	.....	.....	.....	.....	10.7	2.4	14.2	3.9	1.1	19.2
Platanus occidentalis	.....	.....	.....	.....	.....	.....	2.2	8.6	2.2	8.6	4.7	0.7	4.0	9.4
Fraxinus americana	1.1	0.2	2.2	1.0	1.1	1.2	.....	.....	4.4	2.4	4.7	1.5	1.1	7.3
Others	.....	.....	1.1	0.4	.....	.....	.....	.....	1.1	0.4	5.2	0.7	0.5	6.4
Totals	83.9	13.4	107.6	52.4	75.3	109.8	8.7	35.1	275.5	210.7	100.0	100.0	100.0	300.0

area per acre in the 13-24 inch and the 25+ inch diameter classes. Only two saplings and one seedling of this species were found in the areas studied, although seedlings are common nearby in open fields and at the edge of the river. Due to this lack of reproduction and the few individuals in the lower diameter classes, this species will become less important as these forests become more mature.

Acer negundo is third in IVI in areas 1, 2, 4, and 6, due mainly to its high relative frequency. It ranks second in IVI in areas 3 and 5 because of the large number of individuals in the lower diameter classes. This species is much more common than *Populus deltoides* in the 4-6 inch diameter class in all areas studied. No trees of this species were found with a diameter greater than 18 inches. Saplings and seedlings of *Acer negundo* were more common than those of any other species and were well scattered throughout most of the forests. Since this species is able to reproduce in the floodplain forests, indicating some degree of shade tolerance it will probably continue to be relatively important. However, it usually does not attain the size of *Acer saccharinum* and *Populus deltoides,* and its importance will not increase greatly.

Salix nigra is fourth in IVI in areas 1, 2, and 4, and fifth or lower in the others. This species is usually restricted to the lower parts of the floodplain. Often the individuals are clumped and in straight rows, suggesting the river channel was near these areas at one time, and has since changed its course. No seedlings or saplings of this species were found in the forests, but some were found near the river's edge, particularly on sandbars and in association with *Salix interior.* This species will probably die out except near the river's edge.

Ulmus rubra ranks fourth in IVI in areas 3, 5, and 6, and fifth in the remaining areas, due chiefly to its high relative frequency. No trees were found with a diameter greater than 12 inches in five of the areas, and most individuals were in the 4-6 inch diameter class. Saplings were more restricted, however, occurring on natural levees and toward the back of the floodplain near a terrace or hillside where there was less chance of prolonged flooding.

Platanus occidentalis and *Fraxinus americana* are found in some of the areas and usually rank sixth and seventh in IVI. These species are commonly associated with terraced and drier areas and usually do not occur in large number in the typical floodplain forest (Braun, 1950).

The remaining species were scattered and not very abundant. In most cases, these were found near the terrace or hillside limiting the back of the floodplain and in higher areas where it is slightly drier and there is less chance of prolonged flooding. These species are more characteristic of small creeks where flooding is not a common occurrence.

A total of 215 dead-standing individuals were found with a total basal area of 69.62 square feet. Of these, 22 were *Acer negundo* with a total basal area of 4.08 square feet; 83 were *Acer saccharinum* with a total basal area of 24.78 square feet; 52 were *Populus deltoides* with a total basal area of 13.52 square feet and 49 were *Salix nigra* with a total

TABLE 7.—Average Number of Seedlings and Saplings Per Acre in The Six Floodplain Forests Studied, and The Average Per Acre for All Areas Combined.

Species	Average Number of Seedlings Per Acre							Average Number of Saplings Per Acre						
	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Average All Areas	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Average All Areas
Acer negundo	46.2	28.9	40.9	23.3	7.8	32.3	29.9	22.5	46.1	38.4	42.1	172.7	40.9	60.5
Acer saccharinum	4.2	4.7	18.1	4.2	1.3	32.3	10.8	44.5	19.4	41.8	29.1	9.1	45.2	31.5
Ulmus americana	18.6	4.7	21.6	2.9	18.8	34.4	16.8	23.6	1.7	23.7	2.6	20.8	17.2	14.9
Celtis occidentalis	14.6	12.1	.....	11.3	13.0	92.5	23.9	3.1	2.6	0.4	2.6	4.5	12.9	4.3
Morus rubra	4.2	2.6	4.3	4.5	2.6	9.7	4.7	2.0	0.4	3.9	1.9	2.6	2.2	2.2
Fraxinus americana	4.2	1.7	4.3	0.3	.....	4.3	2.5	2.3	0.4	1.7	.....	3.2	6.5	2.3
Others	2.5	3.9	0.9	3.2	4.5	15.1	5.0	0.3	.....	1.7	0.9	2.6	4.3	1.6
Totals	94.5	58.6	90.1	49.7	48.0	220.6	93.6	98.3	70.6	111.6	79.2	215.5	129.2	117.3

basal area of 23.27 square feet. Also, two large stumps of *Acer saccharinum* and three of *Populus deltoides* were found in area 2. These were included in the dead-standing totals. Most of the dead individuals were scattered throughout the floodplain forests. However, dead-standing individuals of *Salix nigra* showed definite clumping, usually associated with old river channels.

The average number of seedlings (more than 1 foot tall and less than 1 inch d.b.h.) and saplings (1-4 inches d.b.h.) per acre in the six areas studied are shown in Table 7. Seedlings less than 1 foot tall were not recorded due to the dense herbaceous understory. The species with the most seedlings and saplings were *Acer negundo, Acer saccharinum, Ulmus americana, Celtis occidentalis, Morus rubra,* and *Fraxinus americana,* in that order of abundance. These were especially common on higher areas and in more open parts of the forests. *Acer negundo* seedlings and saplings were scattered throughout the forests with heavy populations of saplings occurring in open areas. *Acer saccharinum* saplings, in contrast, were mostly found on the natural levees while seedlings were found in open areas and on sandbars at the river's edge. Saplings and seedlings of *Ulmus americana* and *Celtis occidentalis* were also common throughout the floodplain forests. *Ulmus americana* was not represented by trees above 4 inches d.b.h. in the areas studied while *Celtis occidentalis* was very poorly represented. Saplings and seedlings of both species were scattered throughout the forests and exhibited no site preference. A few saplings and seedlings of *Morus rubra* and *Fraxinus americana* were scattered throughout the floodplain and occasional individuals of *Juglans nigra, Maclura pomifera, Ulmus rubra, Aesculus glabra, Quercus macrocarpa, Platanus occidentalis, Gleditsia triacanthos,* and *Gymnocladus dioicus* were found. This latter group, which are included under others in Table 7, will probably have little effect on the future composition of the forests until the sites become drier.

Some shrubs and vines were found in the floodplain forests. These included the shrubs *Sambucus canadensis, Salix interior, Rhus radicans, Staphylea trifolia, Lindera benzoin, Euonymus atropurpureus,* and the vines *Campsis radicans, Parthenocissus quinquefolia,* and *Vitis aestivalis. Sambucus canadensis* averaged about 25 individuals per acre and was scattered throughout the forests with high concentrations in open areas. *Salix interior,* in contrast, was found only on sandbars near the river's edge and in full sunlight with an extensive growth resulting from "root shoots". The remaining shrubs were rarely encountered and were usually associated with higher elevations and open areas.

By far, the most abundant herbaceous species was *Laportea canadensis.* This species grew in all parts of the floodplain forests studied. *Impatiens biflora, Viola papilionacea, Cryptotaenia canadensis,* and *Polygonum virginianum* were also frequently encountered, but not as often as *Laportea.* The distribution of most of the species seems to be dependent upon the moisture in the area and the amount of sunlight which penetrates the overstory. A

complete listing of the herbaceous vascular plants with their distribution and abundance is included in an annotated checklist at the end of this paper.

The results obtained in this study compare favorably with the descriptions of a floodplain forest as described by Braun (1950) except for white elm (*Ulmus americana*). This species was not a significant component of the floodplain forests studied. However, red elm (*Ulmus rubra*) was important and ranked as high as fourth in IVI. The absence of white elm was probably due to phloem necrosis and Dutch Elm disease which killed many of the elm trees about fifteen years ago.

The floodplain forests studied are mixed soft-hardwood according to Hosner and Minckler (1963). In their successional studies of this type of floodplain forest in Southern Illinois, *Salix nigra, Salix interior,* and *Populus deltoides* represent the pioneer species. Soon *Platanus occidentalis, Acer saccharinum,* and *Acer negundo* seedlings appear, but since these species grow slower, they are pole-sized or smaller as *Salix nigra* and *Populus deltoides* approach biological maturity. They further state that the frequency of *Acer negundo* and *Acer saccharinum* suggests that the next stand will consist predominantly of these two species. The results obtained in the present study suggest that these forests are progressing toward the *Acer saccharinum-Acer negundo* stage, and that *Salix nigra* and *Populus deltoides* will become less important. This is indicated by the large number of dead-standing individuals of *Salix nigra,* the small number of lower diameter individuals of *Populus deltoides,* and the lack of seedlings and saplings of both species in the forested areas.

Acknowledgments

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Annotated Checklist of Vascular Herbaceous Plants of the Embarras Floodplain Forests with Their occurrence and General Distribution

The total number of harbaceous taxa of vascular plants recorded from the Embarrass floodplain forest areas was 51. None of these were fern or fern-allies, while 8 were monocots and 43 were dicots. In the following list, the nomenclature follows that of Jones (1963). Each species is followed by its general distribution and occurrence in the areas studied, the collector's name, and collecting number. All specimens are deposited in the herbarium of Eastern Illinois University.

Acanthaceae
- *Ruellea strepens* L. Opening near back of Area Two; rare. Ebinger 7888.

Amaranthaceae
- *Amaranthus hybridus* L. Openings in Areas Two and Three; Ebinger 7878.

Araceae
- *Arisaema dracontium* (L.) Schott. Shaded, higher parts of all areas; rare. Observed.

Balsaminaceae
- *Impatiens biflora* Walt. Throughout the floodplain forests; frequent to locally common. Observed.
- *Impatiens pallida* Nutt. Mostly near back of Area Five; locally common. Crites 501.

Campanulaceae
- *Campanula americana* L. Openings on levees; rare. Crites 519.

Chenopodiaceae
- *Chenopodium album* L. Opening at back of Area Two; rare. Ebinger 7927.

Compositae
- *Ambrosia trifida* L. Opening in Area Two; locally common. Ebinger 7944.
- *Aster ontarionis* Wieg. Scattered throughout the floodplain forests; common. Ebinger 7933.

Eupatorium coelestinum L. Openings on levees; rare. Crites 502.
Eupatorium rugosum Houtt. Openings on levees; rare. Crites 512.
Rudbeckia laciniata L. Scattered throughout the floodplain forests; occasional to locally frequent. Crites 511.
Verbesina alternifolia (L.) Britt. Drier parts of Areas Five and Six; rare. Ebinger 7935.

Convolvulaceae
Cuscuta gronovii Willd. Growing as a parasite on *Laportea* in Area Two; occasional. Ebinger 7891.

Cruciferae
Iodanthus pinnatifidus (Michx.) Steud. Drier parts of Areas Two and Three; rare. Ebinger 7609.

Cucurbitaceae
Sicyos angulatus L. Vine growing in shaded parts of most areas, occasional. Ebinger 7887.

Cyperaceae
Carex grayii Carey. Low, wet parts of floodplain forests; rare. Crites 515.

Euphorbiaceae
Acalypha deamii (Weatherby) Ahles. Opening on levee in Area Four; rare. Ebinger 7941.
Acalypha rhomboidea Raf. Openings in most areas; occasional. Ebinger 7882.
Acalypha virginica L. Opening at back of Area Two; rare. Ebinger 7930.
Chamaesyce maculata (L.) Small. Opening near back of Area Two; rare. Ebinger 7931.

Gramineae
Cinna arundinacea L. Scattered throughout most areas; occasional. Ebinger 7929.
Elymus villosus Muhl. Scattered throughout most of the floodplain forests; occasional. Ebinger 7936.
Elymus virginicus L. Shaded areas; occasional. Crites 504.
Glyceria striata (Lam.) Hitchc. Low, wet parts of Area Six; rare. Crites 510.
Leersia virginica Willd. Moist, open areas; locally common. Ebinger 7946.
Muhlenbergia frondosa (Poir.) Fern. Openings on levess; rare. Ebinger 7880.

Labiatae
Lycopus virginicus L. Drier part of Area Three; rare. Ebinger 7934.
Scutellaria lateriflora L. Moist part of Area Four; rare. Ebinger 7942.
Stachys tenuifolia Willd. Opening near back of Area Two; rare. Ebinger 7890.

Leguminosae
Amphicarpa comosa (L.) G. Don. Vine growing in shaded areas; rare. Ebinger 7883.

Polemoniaceae
Phlox paniculata L. Openings in Area Six; locally frequent. Crites 509.

Polygonaceae
Polygonum scandens L. Openings on levees; occasional. Ebinger 7886.
Polygonum pensylvanicum L. Openings on levees; rare. Ebinger 7885.
Polygonum punctatum Ell. Openings on levees; rare. Ebinger 7881.
Polygonum virginianum L. Scattered throughout the floodplain forests; common. Ebinger 7928.
Rumex verticillatus L. Wet areas; rare. Crites 508.

Ranunculaceae
Delphinium tricorne Michx. Heavily shaded areas; rare. Crites 506.
Ranunculus abortivus L. Wet, shaded areas; rare. Crites 507.
Ranunculus septentrionalis Poir. Wet, shaded areas; rare. Crites 505.

Rosaceae
Geum canadense Jacq. Near back of Area Five; occasional. Ebinger 7938.

Scrophulariaceae
Chelone obliqua L. Small colony in open part of Area Five; rare. Ebinger 7940.

Solanaceae
Physalis pendula Rydb. Openings on levees in Areas Five and Six; rare. Ebinger 7874.
Solanum nigrum L. Openings on levees in Areas Five and Six; rare. Ebinger 7884.

Umbelliferae
Cryptotaenia canadensis (L.) DC. Scattered throughout the floodplain forests; common. Crites 514.
Sanicula gregaria Bickn. Scattered throughout the floodplain forests; occasional. Crites 513.

Urticaceae
Boehmeria cylindrica (L.) Sw. Near back of Area Two; rare. Ebinger 7889.
Laportea canadensis (L.) Gaud. Throughout the floodplain forests; abundant. Crites 503.
Pilea pumila (L.) A. Gray. Scattered throughout the floodplain forests; frequent. Ebinger 7945.
Urtica gracilis Ait. Scattered through-

out the floodplain forests; occasional. Ebinger 7879.

Violaceae

Viola papilionacea Pursh. Scattered throughout the floodplain forests; abundant. Crites 517.

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NOTES

A NEW MULTIPLE-PLATE SAMPLER FOR COLLECTING MACROINVERTEBRATES OF THE STREAM BIOCIES

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ABSTRACT.—The construction and use of a new sampling device which can be used to analyze quantitatively faunal populations of swift water areas of streams is described.

The ever increasing aesthetical and recreational demands on our freshwater streams today and the pollution problem make obvious the need of reclaiming polluted streams and increasing the beauty and productiveness of non-polluted streams.

A knowledge of the kinds and numbers of macroinvertebrates which inhabit a stream is important as they are vitally related—as food chain organisms —to its production of fishes. The absence, sparsity or abundance of these animals may also be used as indicators of the physical as well as the chemical quality of a stream. Results of comparative productiveness studies, determination of degree of pollution and potential productiveness of various streams or different stretches of the same stream can be used to program and institute stream improvements. Because much data for comparisons is needed, we must have a better method than the classical ones used for sampling invertebrates of stream communities. These older, conventional sampling methods —as the Peterson dredge and Surber sampler—are toilsome and seem to be inadequate for studies in deeper swift waters, on rocky bottoms, and in stream areas where fallen logs, limbs and brush are lodged.

This need of a more effective and efficient sampling method for analyzing benthic macroinvertebrates of riffle stream areas challenged the authors to devise a new sampler. The new sampler is an outgrowth of the multiple-plate samplers used by Hester and Dendy, which they employed in studies of benthic macroinvertebrates in ponds (Hester and Dendy, 1962). Where rigidity to withstand current is not essential in ponds, they used a nylon cord hanging from a branch of a tree to suspend the sampler units on the bottom. When they used their samplers in streams, each was fastened to a vertical wire that extended from a heavy weight on the bottom to an overhanging tree. At a distance from the weight, the wire was wrapped around the neck of the assemblage bolt to maintain the sampler at a selected depth. Their procedure necessitated an advantageously located tree with a limb overhanging the sampling site for the suspension wire attaching to the sampler, and even though the samplers were attached by the wire, they would sway and vibrate unnaturally in the current when used in swift water of riffles. Also the samplers were disturbed many times by curious bypassers who saw the exposed wires. The overall accuracy of sampling results obtained using this arrangement is doubtful. Some modifications of the multiple-plate sampling methods of Hester and Dendy, mainly the attachment of a contrivance to stabilize the samplers in swift waters and a different method—in lieu of their

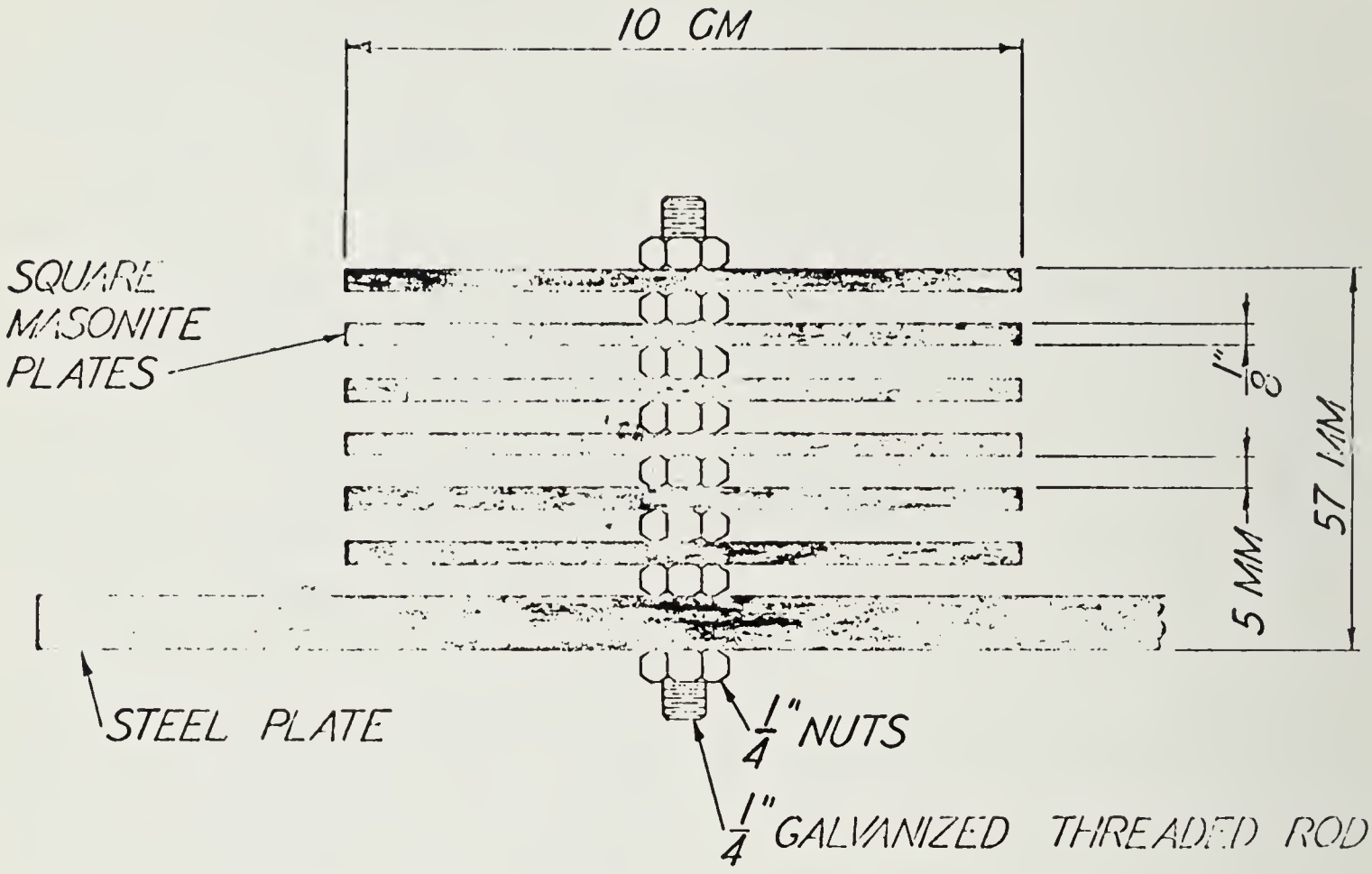

FIGURE 1.—Diagram showing side view of a riffle multipleplate sampler attached to basal steel plate. Size measurements of structural parts which are standard commercial products have been purposely labeled in inches rather than converting them to the metric system.

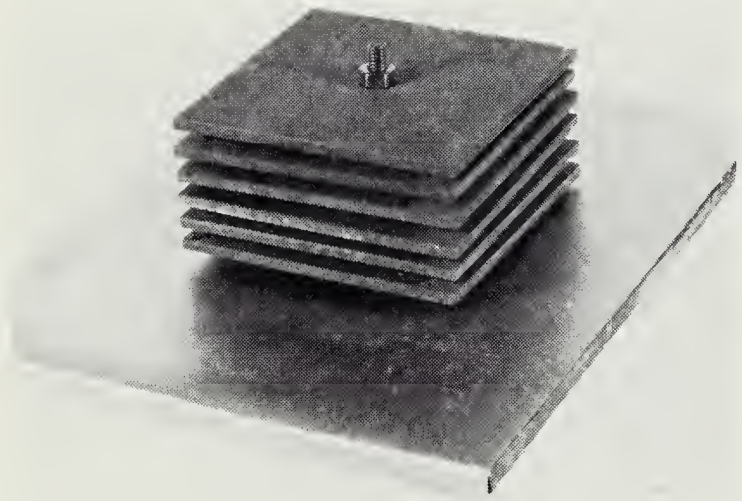

FIGURE 2.—Photograph of assembled riffle multple-plate sampler, showing sampler unit with attached basal steel anchor plate.

use of plastic bags—for retrieving the samplers from the water were, therefore, made. The new sampler has been named a "riffle multiple-plate sampler."

The newly devised sampler consists of one-eighth inch thick tempered hardboard (Masonite) which was cut into 10 cm square plates. A nine thirty-second inch hole was drilled in the center of each plate. Six of these plates were placed on a one-fourth inch galvanized threaded rod. The plates were separated by hexangular nuts, which were 5 mm thick. Thus, the plates were spaced 5 mm apart, an ample space for macroinvertebrates to move freely between the plates, and still offer them a protected microhabitat from larger predators. The total area per sampler unit, consisting of the six plates, was 1200 square cm. Each sampler assemblage was attached by the threaded rod and a nut to a heavy basal steel plate, 9 x 7½ x 5/8 inches, which weighed 7 pounds and 12 ounces (Figs. 1 and 2). This size basal plate was adequate to hold the samplers stationary in swift water. The dimensions and weight of the steel plate is, however, abritrary. The plate, of course, must be heavy enough to hold the sampler unit stable in swift water. By coating the steel plates and the nuts with Fiberglas, rusting was prevented.

During sampling studies, to prevent the loss of any of the organisms attached to the sampler units, each was removed from the water by using a 3-pound coffee can. The sampler was approached from downstream and the

open end of the coffee can was placed over the sampler unit tightly down onto the steel plate. The steel plate with attached sampler unit, and coffee can with water were then inverted. The steel plate was removed from the sampler unit at the collecting site. The coffee can with contained sampler and water was covered with a plastic lid and brought back to the laboratory. These procedures insured a capture of all attached organisms. At the laboratory, the sampler plates were disassembled in some water in a white porcelain pan, and while observing through binocular microscope, the invertebrates attached to the plates and those dislodged in the pan were removed by hand picking and counted. The remaining water in each coffee can was strained through a pair of brass testing sieves. The first sieve had a mesh size of one mm, while the second had a mesh size of 500 microns. Any invertebrate that did not pass through the smaller sieve was considered a macroinvertebrate in the studies. This procedure enabled collection and counting of fauna which had become detached during handling and transporting.

The new riffle multiple-plate sampler eliminates former disadvantages by the attachment of the heavy steel basal plate to the sampler unit for anchorage. There is no need of a tree for its attachment; it is completely stabilized in the swift water of the riffle, eliminating swaying and vibration; and it is not easily discovered by bypassers, because the entire sampling unit is submerged. Further, the sample plates can be easily removed from the water by the use of a 3-pound coffee can—rather than having to manipulate a plastic bag — to enclose them in the swift water. The new samplers can be used to qualitatively and quantitatively study microinvertebrates as well as macroinvertebrates. They are economical, simple to construct, convenient to transport, and easy to place and retrieve from the stream.

Obvious shortcomings of the riffle samplers are that some time must elapse before an accurate sample can be taken, unlike when using the Peterson dredge or Surber sampler, and that the artificial substrate collects only non-burrowing invertebrates.

The samplers have been used with much success at Northern Illinois University in the study of stream ecology: 1) to compare, qualitatively and quantitatively, benthic macroinvertebrate populations of riffles of various streams of northern Illinois, 2) to show population fluctuations of macroinvertebrates of riffle areas during a 12 month period, 3) to determine intimate, microhabitat preference of macroinvertebrates of the riffle by altering the surface texture of the plates, and 4) to compare the macroinvertebrate populations of the riffle stream areas and the sand-bottomed areas. In the latter study we used the sampler plates as simulated substrate objects in the riffle and sand-bottomed areas. A comparison of the populations collected in the two different areas gave us, therefore, the productive potentialities of a stream when substrate objects as those found in the riffle area are also placed in the sand-bottomed area. The results were conclusive. They showed—that if artificial placement of stream improvement objects as rocks, concrete slabs, logs and timbers in sand-bottomed areas—productiveness of a stream may be more than doubled and maintained more uniform and stable throughout the entire year.

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Manuscript received October 24, 1968.

IDENTITY OF A SHORT-TAILED WEASEL FROM CENTRAL ILLINOIS

CHARLES A. LONG
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ABSTRACT.—A weasel reported as an ermine was misidentified.

Long (1968) identified a road-killed, lactating weasel from Bondville, Illinois, as *Mustela erminea*, but the specimen (WSUM-28) is referable to *M. frenata*. Although the skin is small (total length, 261 mm) and the tail very short (only 66 mm, and normal), the feet are all brown as in *frenata*. The smashed skull when reconstructed showed a postglenoidal length of approximately 50 per cent of the condylobasal length (resembling *erminea*), but the latter measurement might have been longer than it appears and is longer than usual for *erminea*. One remaining postorbital process is pointed as in *frenata* (see Burt, 1948). The brown feet and pointed process outweigh the characters of small size, short tail, and high ratio of postglenoidal to condylobasal lengths that are usually listed as diagnostic for the ermine (Hoffmeister, 1950; Hoffmeister and Mohr, 1957; Hall, 1951) especially inasmuch as the ermine is unknown in Illinois.

Long's paper (1968) also has a printing error listing the catches of *Blarina* as 13 instead of the range 1-3.

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Manuscript received October 30, 1968.

EFFECT OF CYCLOHEXIMIDE ANALOGS ON THE GROWTH OF MICROORGANISMS

BERNADINE F. PELETZ and M. J. STARZYK
Northern Illinois University, DeKalb

ABSTRACT.—The effect of cycloheximide (Acti-dione) analogs on the growth of bacteria, yeasts and fungi was studied. These analogs had no effect of the bacteria or yeasts tested but did demonstrate variable activity against the fungi. Most notable was the inhibition of the plant pathogen, *Diplodea zeae* by 4-desmethyl dihydroxycycloheximide (test compound no. 6).

The inhibitory action of cycloheximide (β-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide) against many plant pathogens is well known. Numerous analogs of this compound have been recently synthesized in a search for chemicals possessing antitumor activity. It was the purpose of this project to test these compounds against representative bacteria, yeasts and molds and determine whether their particular structural features would produce any inhibitory effect.

METHODS AND MATERIALS

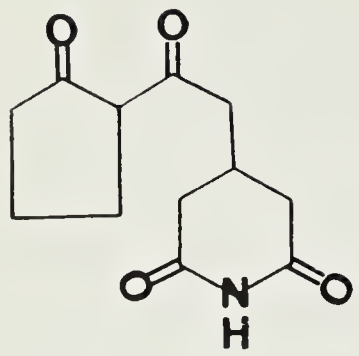
Inhibitory activity against bacteria and yeasts was evaluated by the paper disc-seeded agar method. Bacteria were grown in nutrient broth and yeasts were grown in peptone (10 g/liter) dextrose (20 g/liter). After appropriate growth had occurred, 10 ml. of an organism was aseptically pipetted into 100 ml. of melted nutrient agar (bacterial) or peptone dextrose agar (yeasts. The seeded agar was dispensed into Petri dishes (90 mm in diameter) at a volume of 10 ml. per plate. Test solutions (see figure 1) were prepared by dissolving the chemicals in sufficient ethanol to produce a 1000 ug/ml. solution. Paper discs (Whatman assay Discs, 12.7 mm in diameter) were dipped into the test solutions, allowed to dry and then placed upon the seeded agar plates. The test plates were then incubated at 25°C. or 37°C. depending on growth requirements. The plates were examined on each of the following five days for inhibition zones surrounding the treated discs.

Inhibitory activity against molds was evaluated by incorporation of an aqueous solution of the test compound into potato dextrose agar to give a final concentration of 100 ug/ml. in the treated agar. Three plates of this concentration as well as three control plates were prepared for each fungus as well as each compound tested. Approximately 15 ml. of agar was added to each Petri dish and allowed to solidify. Five millimeter sections of fungus mycelium were secured from actively growing fungus cultures by using a sterile #2 cork-borer. Each section was placed in the center of a dish with the mycelium side down on the treated agar. All plates were incubated at room temperature and were examined for growth after 72 hours.

RESULTS AND DISCUSSION

Of the ten analogs tested, none demonstrated any activity against the following yeasts and bacteria: *Saccharomyces pastorianus*, *Endomycopsis bispora*, *Endomycopsis fibuliger*, *Saccharomyces cerevisiae*, *Hansenula anomala*, *Citeromyces matritensis*, *Schizosaccharomyces octosporus*, *Trichosporon roseus*, *Rhizopus stolonifer*, *Escherichia coli*, *Micrococcus luteus*, *Proteus vulgaris*, *Serratia marcescens* and *Aerobacter aerogenes*.

Only six of the analogs were tested against the fungi because of various insolubility problems. From the data in Table 1 it can be seen that compound



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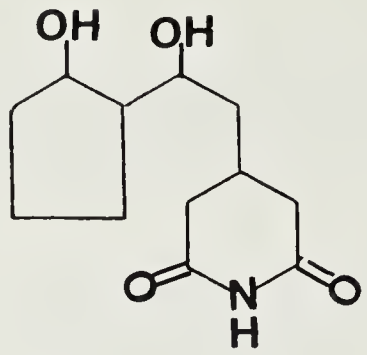

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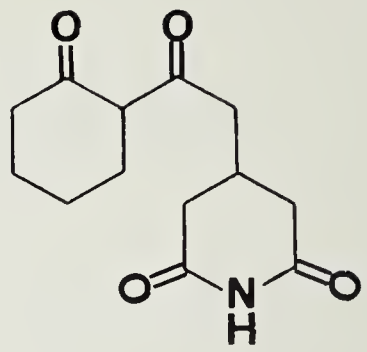

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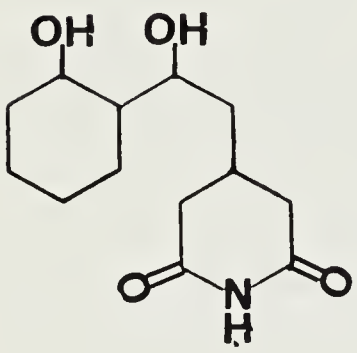

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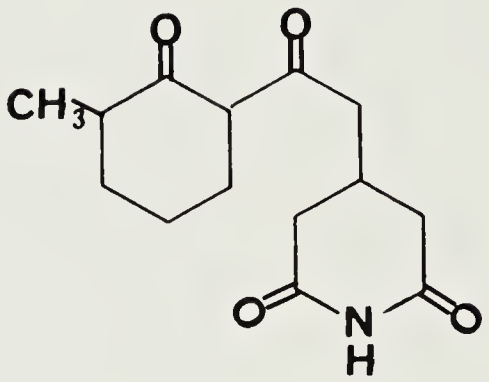

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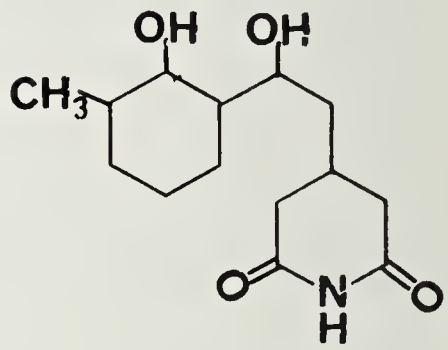

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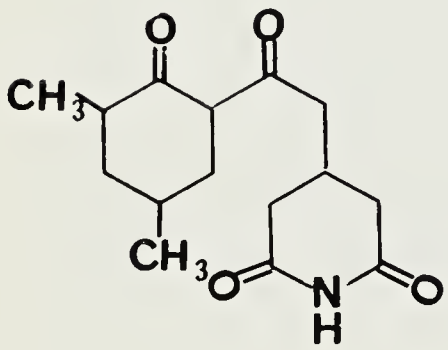

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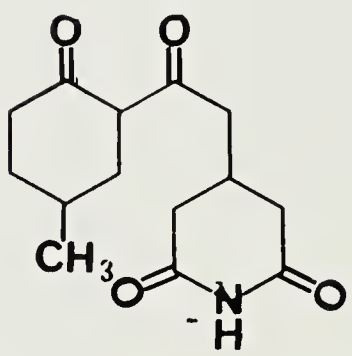

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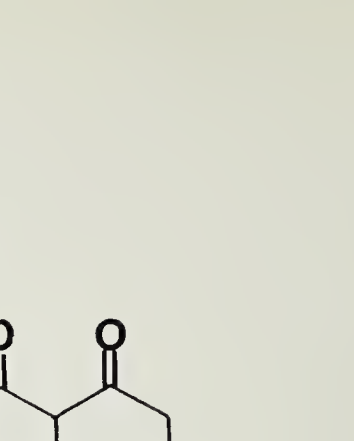

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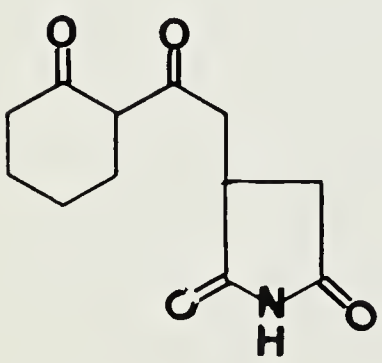

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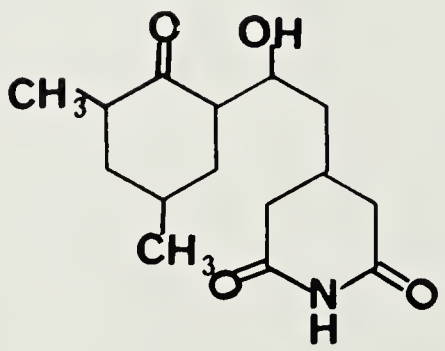

Cycloheximide

Figure 1. Analogs of Cycloheximide

TABLE 1. Percent inhibition of growth at 25°C. in relation to controls.

Fungus	Compound tested						
	1	3	4	5	6	10	control
Sclerotinia sclerotiorum	20.2	19.5	24.2	29.0	20.9	23.7	0
Curvularia sp.	7.1	13.9	6.1	28.9	33.8	10.2	0
Rhizoctonia solani	4.8	3.1	2.9	24.0	29.8	25.3	0
Alternaria solani	4.3	2.2	4.9	16.0	17.3	15.4	0
Diplodia zeae	9.8	1.9	7.0	39.4	70.4	34.5	0
Chaetomium coprinum	27.2	17.6	2.1	12.1	7.9	12.1	0
Cerotocystis fagacearum	13.4	9.4	7.6	9.9	15.2	4.7	0
Colletotrichum circans	14.2	25.3	15.8	20.0	2.6	12.1	0
Ceratocystis ulmi*	0	0	0	0	0	0	0

* For *Ceratocystis ulmi* there was a slight stimulatory response in growth to all the test chemicals in relation to the control. Since percent inhibition is recorded, no value other than 0 is appropriate here.

no. 6 (4-desmethyl dihydroxycycloheximide) was definitely active against *Diplodia zeae*. Except in the case of *Ceratocystis ulmi*, all compounds did have a slight inhibitory effect on the filamentous fungi tested.

ACKNOWLEDGMENTS

Partially supported by grants from the American Cancer Society.

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Manuscript received December 10, 1968.

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ALICE LOVINA KIBBE

1881-1969

EVERETT F. MORRIS
Western Illinois University, Macomb

Alice Lovina Kibbe was born in Bridgewater, South Dakota, on June 27, 1881. Her early years on the Dakota prairies instilled in her a love of nature and a desire to protect and preserve it. She attended Baptist College in Sioux Falls, South Dakota, and then at the age of seventeen taught in a country school.

Dr. Kibbe's father, a newspaper editor, moved his family to the state of Washington, and Miss Kibbe attended the State Normal in Bellingham from which she was graduated in 1905. Her undergraduate education was continued at the University of Washington where she received the A.B. degree in 1910. From 1910-1913, Miss Kibbe was a teacher and principal at Winlock, Washington. She spent her summers at the Marine Biological Station of the University of Washington at Friday Harbor. She received the M.A. degree from the University of Washington in 1914. Miss Kibbe served as a teacher and principal at Mossy Rock, Washington from 1914-1917. During the 1917-1918 school year, she served as Superintendent of Schools in Adna, Washington. She returned to teaching and at the end of World War I was the only woman in the nation in charge of Smith-Hughes Agriculture. Miss Kibbe received a M.S. degree from Cornell University in 1920. She became Professor of Biology and Head of the Dept. of Biology at Carthage College in 1920. Professor Kibbe received the Ph.D. degree from Cornell University in 1926. Her doctoral dissertation was "A Plant Survey of Hancock Co., Ill." Dr. Kibbe remained a Professor and Dept. Head at Carthage College until 1956. From 1956 until 1964 when the Illinois campus of Carthage College was closed, Dr. Kibbe served as Curator of the Mu-

seum and Herbarium at Carthage College.

Dr. Kibbe was a woman of remarkable energy. Some of her efforts during the 44 years she lived in Carthage were directed towards the establishment and maintenance of a museum at the College. Memorabilia of the area's past were placed in the museum. When the college moved to a new campus in Kenosha, Wisconsin, Dr. Kibbe purchased a great portion of the items from the college. In 1964, she offered the museum items and her home in Carthage to the city as a museum. Her offer was accepted and the Alice L. Kibbe Museum was opened in 1964. A tract of about seven acres, two miles from Carthage, was obtained by Dr. Kibbe in 1921. The area was subsequently used by Dr. Kibbe and her students as a wildlife preserve. The preserve was presented to the city of Carthage by Dr. Kibbe with the provision that the Community Garden Club of Carthage supervise the care of the area. It is now known as the Kibbe Wildlife Preserve.

With the purchase of fifty-four acres of land along the Mississippi River at the edge of Warsaw, Professor Kibbe established the Mississippi River Science Station in 1932. The Station was used principally for field trips and weekend camp-outs by Carthage students and faculty since that time. Through subsequent purchases of adjoining properties, the Station grew to approximately 200 acres. At the closing of the campus in Carthage in 1964, Dr. Kibbe deeded the Science Station to Western Illinois University. The Board of Governors of State Colleges and Universities in Illinois designated the Station as the Alice L. Kibbe Life Science Station and since 1964 it has been in operation as a teaching and research facility of Western Illinois University.

The Kibbe Collection along with the remainder of the Carthage Herbarium was moved to the new campus of Carthage College in 1964. A few hundred unmounted specimens were presented to Western Illinois University by Dr. Kibbe and these have been placed in the Herbarium of the A. L. Kibbe Life Science Station.

Dr. Kibbe is best known for her "A Botanical Study and Survey of a Typical Mid-Western County (Hancock County, Illinois)." The book, mimeographed and hardbound, published by the author in 1952, is a classic. It represents her doctoral dissertation completed in 1926 plus additional work carried out in the ensuing years. A second book, published in the fashion of the first, and titled "Afield with Plant Lovers and Collectors (Patterson correspondence with greatest American Botanists 1870-1915)" appeared in 1953. The volume is rich in botanical history of the era.

Based on earlier studies, Dr. Kibbe published "Some Points in the Structure of *Alaria fistulosa*" in 1915 in the Puget Sound Marine Sta. Pub. (1:43-57) and "*Chytridium alarium* on *Alaria fistulosa*" in 1916 in the Puget Sound Marine Sta. Pub. (1:221-226). The former traced the development of the fistulose or chambered character of the midrib of the kelp, *Alaria fistulosa.* The latter named and described a new fungus species, *Chytridium alarium,* and discussed its life history.

In one of her last professional appearances, Dr. Kibbe, at the age of eighty, read a paper on the "Effects of the Mississippi River Scenic Highway Program on Wildlife" before the Conservation Section during the 54th Annual Meetings of the Illinois State Academy of Science at Charleston in 1961.

Dr. Kibbe was very companionable and ever helpful to her students. She took great personal interest in each of them. Through the years she kept in touch with her former students and friends by means of her Christmas messages. They will be greatly missed.

In 1964, Dr. Kibbe left Carthage and Illinois in order to live her remaining years with relatives in Bellingham, Washington. On January 21, 1969, Dr. Alice L. Kibbe passed away. She was buried in Elma, Washington.

Manuscript received, February 21, 1969.

SKY CONDITIONS IN RELATION TO RING-BILLED AND HERRING GULL ORIENTATION

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ABSTRACT. — The homing behavior of 379 Ring-billed and 50 Herring Gulls was contrasted with sky conditions that existed at release times. No evidence was obtained that supported the use of sun or stars as orientational cues. Gulls responded to topographical features during some trials. Juvenile Ring-billed Gulls showed directional preferences that correspond to headings required to reach the population's winter range.

This paper discusses one aspect of a study designed to investigate the orientation and migration of Ring-billed Gulls (*Larus delawarensis*) and Herring Gulls (*L. argentatus*) from a single breeding colony at Rogers City, Presque Isle County, Michigan.

MATERIALS AND METHODS

During the summers of 1963 and 1964, 320 adult, 3 subadult, and 56 juvenile Ring-billed Gulls and 50 adult Herring Gulls were used in 429 homing trials. Forty-one of the Herring Gulls were equipped with small transmitters and tracked for a combined total of 1302 miles with direction-finding receivers mounted in two vehicles. In addition, 294 Ring-billed Gull chicks, aged 1 to 20 days, and two Herring Gull chicks were subjected to orientation cage experiments (during 1964 *see* Southern, 1967a and 1969a).

Experimental gulls were released under varying amounts of sky cover, during winds from several directions, at different times of the day and night, in several directions from the colony, adjacent to major topographical features (*e. g.*, large bodies of water), and at various distances (2 to 150 miles) from the colony. Whenever possible birds of known age and sex were used. One to ten individuals were released at a time.

As controls 49 Ring-bills were released in or near the colony in an attempt to determine: (1) the accuracy of visual methods for calculating return times of color-marked gulls; (2) the effect of handling and confinement on return rates; and (3) the probability of recording color-marked individuals during any one visit to the colony.

Most of the adult Ring-billed Gulls were captured by means of a cannon net trap (Southern, 1967b). This method was not suitable for catching Herring Gulls because of their wariness and the relatively wide spacing of their nests. Adults of this species were snared with nylon loops placed on nests or perches. Each gull received a numbered band and was color-marked with alcohol-soluble stains for individual recognition.

As an improvement in handling, during 1964 the birds were given intramuscular injections of Equitol (supplier, Jensen-Salisbery Laboratories) at the rate of 20 ml/kg to quiet them during shipment and thereby reduce the chances of overheating. When ambient temperatures were below 88° F this dosage immobilized gulls for about two hours during the daytime; at night and at higher temperatures this amount was occasionally fatal. The gulls were put in burlap bags and carried to release sites by automobile. On the basis of my observations, the orientation of drugged birds was not different from that of the undrugged released in 1963. Controls released near the colony also received injections and their behavior supported this generalization.

Sky conditions at release times were recorded and also the changes that occurred during trials for comparison with concurrent homing success rates, speeds, and initial departure behavior. Sky conditions were divided into four categor-

ies: (A) cloudless; (B) scattered clouds up to 50 per cent cover; (C) over 50 per cent cloud cover — up to, but before the point where the sun's position was no longer visible to the observer; and, (D) complete overcast. In most instances sky conditions varied during the day, were not consistent for large areas, or were not consistent during the entire time the gulls needed to return. As most gulls took from several hours to several days to traverse return routes, it was impossible to determine whether or not particular sky conditions existed along the bird's entire flight path, except in the case of radio-tracked Herring Gulls (in press, Wilson Bulletin).

The Rayleigh test (Batschelet, 1965) was applied to the data presented in Figures 3, 4, and 6. This test is designed to determine whether the distribution of a population is uniform or whether it is unimodal. If the critical value of the test statistic $z > z_p$, the null hypothesis of a uniform distribution is rejected. If instead $z < z_p$, the assumption $p = 0$ is consistent with the data.

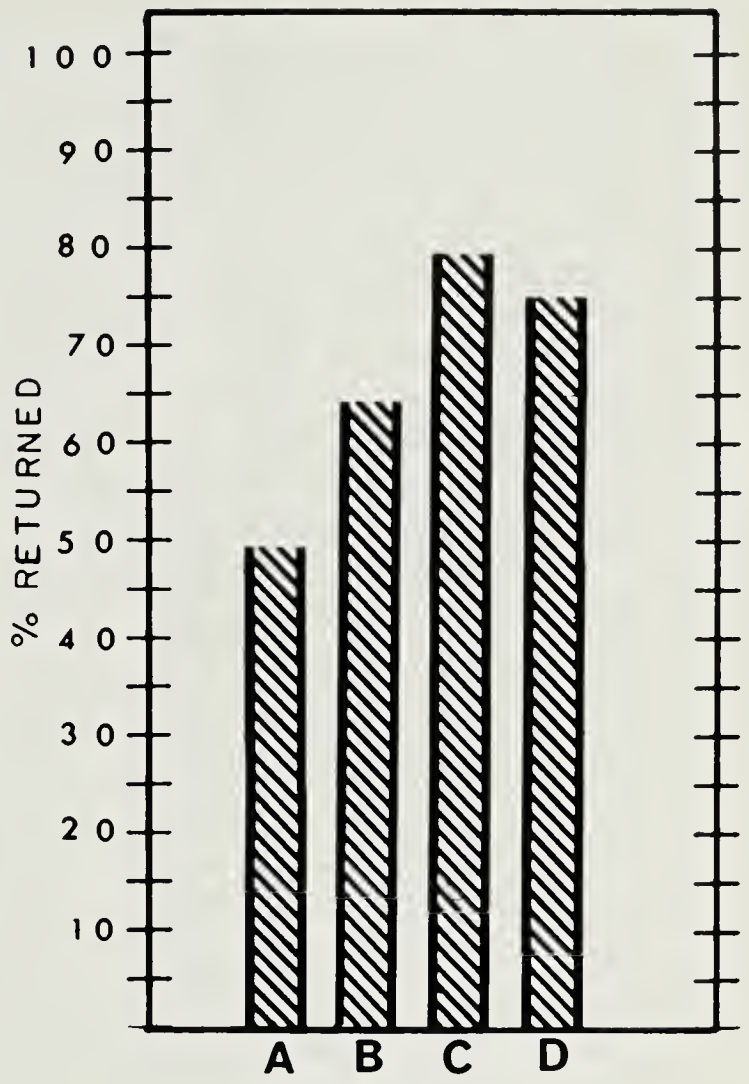


FIGURE 1. Ring-billed Gull returns with reference to sky cover. A = clear sky; B and C = partly cloudy; D = complete overcast. The number of gulls released under sky condition is as follows: A, 39 individuals; B, 99; C, 67; D, 61.

RESULTS

Trials with Adults.

Figures 1 and 2 illustrate the average success rates for Ring-billed and Herring Gulls released 2 to 150 miles from the colony under the four sky conditions. I combined data for the various distances as no variations were apparent. The data indicate that clouds obscuring the sun or stars did not alter homing behavior. Some of the birds released under Sky D may have been able to see the sun at some time during their return flights. As the behavior of radio-tracked gulls released under Sky D was not noticably modified when the sun came out, all of the results from trials initiated during completely overcast conditions were retained and plotted in Category D. The lowest Ring-billed Gull success rate followed diurnal and nocturnal releases under clear skies (Fig. 1). Radio-tracking indicated that gulls followed meandering routes in most instances and responded to particular types of topographical features.

Essentially all of the gulls released at night, regardless of sky conditions at the release locality, failed to return to the colony until several hours after daylight. Radio-tracked individuals spent most of the night resting on the ground or water.

The initial departure headings for adult gulls released under the four sky conditions are plotted in Figures 3 and 4. Categories B and C are combined in these figures as the sun was periodically visible in all instances and because no differences

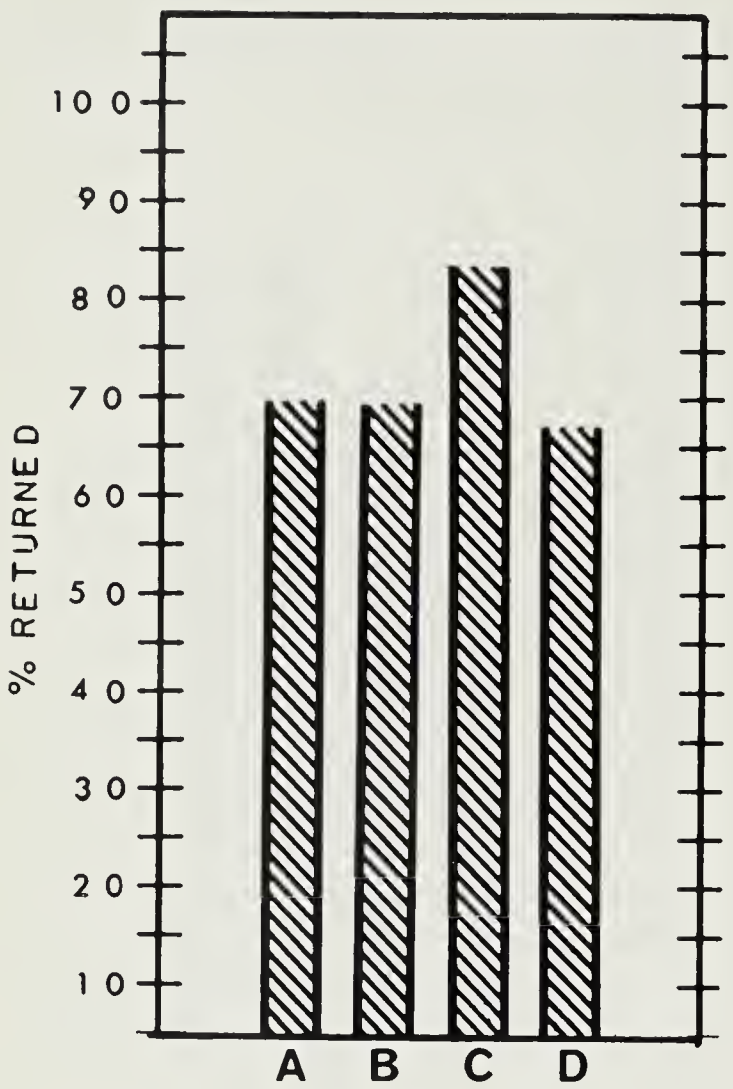


FIGURE 2. Herring Gull returns with reference to sky cover. The number of birds released under each sky category are as follows: A, 10 individuals; B, 13; C, 6; D, 3.

were apparent. Under clear skies (A) only 16.4 per cent of the gulls selected approximately homeward directions for departure (Fig. 3) but 55.1 per cent of the birds released actually homed. At the other extreme, Sky D, 25.0 per cent showed homeward tendencies at departure and 73.5 per cent returned. These results suggest that the presence or absence of solar cues does not influence the selection of departure directions or homing rates.

The mean angle of departure, relative to home, is similar for gulls released 16 to 150 miles from home under each of the three sky types (Fig. 3). In each instance the Rayleigh test shows the mean to be significant at the 5 per cent level since the statistic z is equal to or larger than the z_p value of 3.0 for these sample sizes (Sky A, $z = 4.6$; Sky B-C, $z = 16.1$; Sky D, $z = 3.0$). The difference between the z values for Sky A and Sky D is not considered significant and, therefore, these data are interpreted as indicating that vision of the sun is not essential for the mode of orientation possessed by Ring-billed and Herring Gulls.

At distances under 15 miles, the departure headings form a unimodal distribution (Fig. 4). The sample size is small for Skies A and D but in the latter instance the Rayleigh test shows the mean angle to be significant ($z = 3.2$; $z_p = 2.8$ at the 5 per cent level). The mean direction for Sky B-C is also significant ($z = 7.9$; $z_p = 3.0$).

Figure 5 shows the average homing speeds for two groups of 46 gulls released under clear and overcast skies and at distances between 10 and 110 miles. I was unable to release gulls at sites within each distance category during both clear and overcast conditions and thereby contrast their homing speeds. However, I was able to release birds under Skies A and D at distances up to 75 miles. Comparison of these data showed that gulls released under Sky D had homing rates similar to birds released under Sky A. This further supports my speculation that the sun is not an essential clue for orientation or homing by these species. Furthermore visibility of the sun does not seem to enhance any aspect of the homing performance, i.e., the selection of homeward headings, success rates, or the distance flown.

Trials with Juveniles.

Ring-billed Gull chicks used in

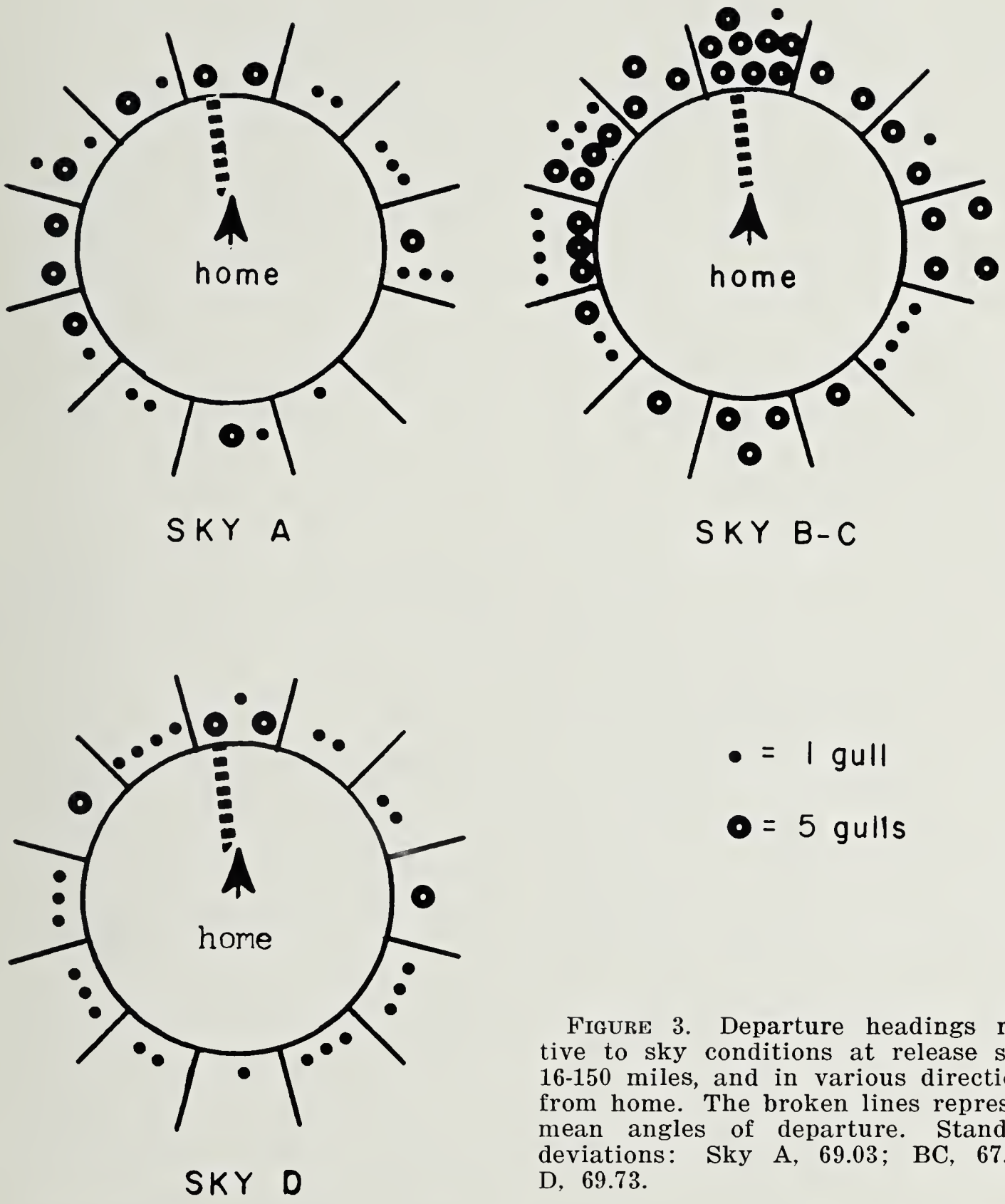


FIGURE 3. Departure headings relative to sky conditions at release sites 16-150 miles, and in various directions, from home. The broken lines represent mean angles of departure. Standard deviations: Sky A, 69.03; BC, 67.67; D, 69.73.

orientation cage trials and juvenile Ring-bills released for their maiden flights at locations 7 to 16 miles from the colony showed preferences for southeast and east directions (Fig. 6). Juveniles occasionally circled for about 20 minutes or longer before selecting a course that finally took them beyond my range of vision. As a sizeable proportion of the young Ring-bills from this population migrate along the Atlantic Coast (Southern, 1967b; 1968), these directional preferences may represent an innate ability to select appropriate migrational headings.

The sun was not apparently involved in these direction preferences by young Ring-bills (Fig. 6). Similar mean angles of flight are evidenced for each sky condition but only those for Skies A and D are

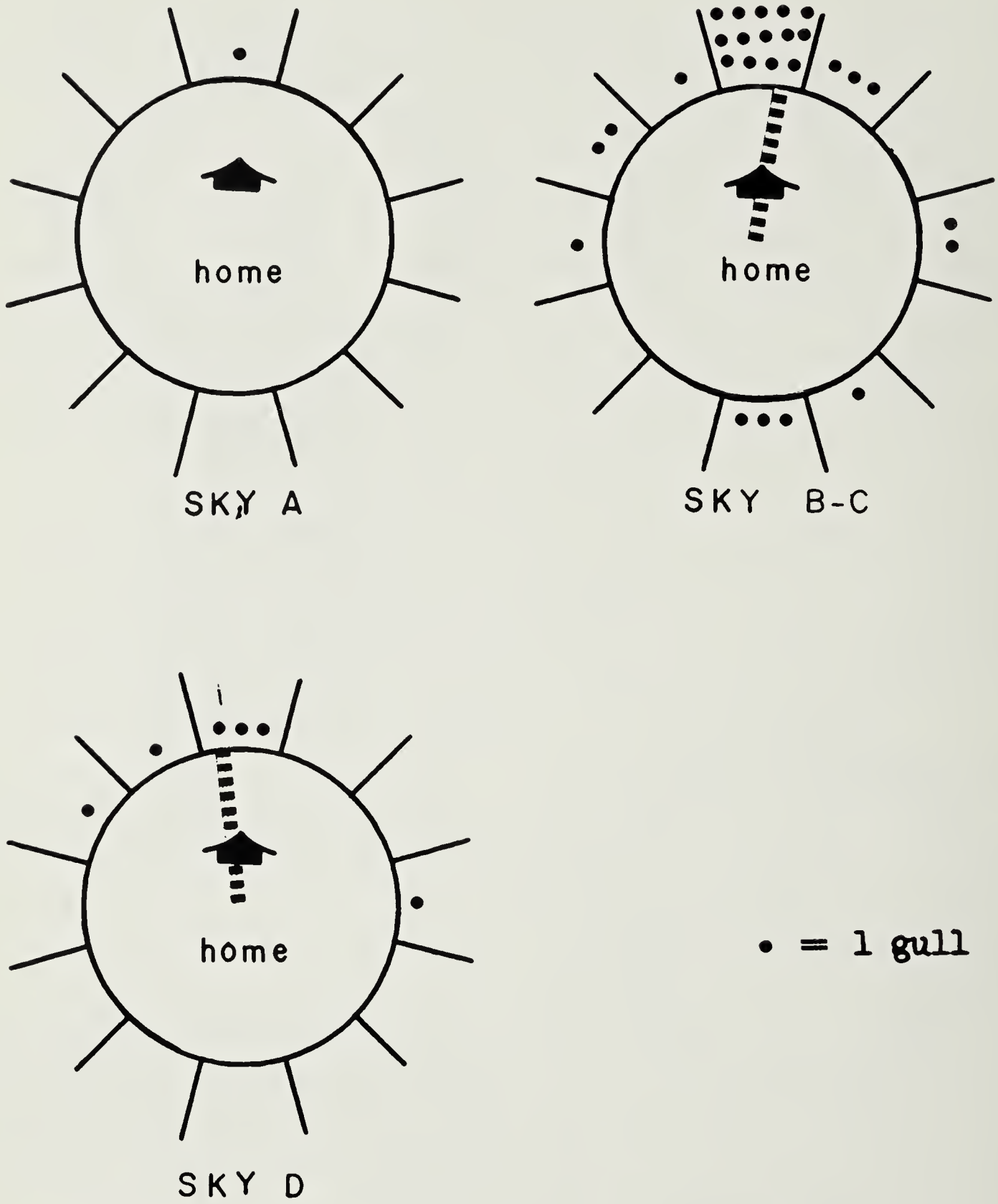


FIGURE 4. Departure headings relative to sky conditions at release sites 1-15 miles, and in various directions, from home. Broken lines represent the mean angles of departure. Standard deviations: Sky A, 0.0; BC, 54.87; D, 42.09.

significant (A, $z = 6.39$, $z_p = 2.95$; B-C, $z = 0.20$, $z_p = 2.93$; D, $z = 4.75$, $z_p = 2.95$). It seems, therefore, that the ability to select east or southeast courses during trials, and possibly upon dispersal from the colony, is based on some mechanism other than the use of solar cues.

DISCUSSION

The data presented herein indicate that departure directions of Ring-billed and Herring Gulls from the Rogers City population are not affected by cloud cover although flight patterns and general behavior may be (*e. g.*, the gulls flew at lower al-

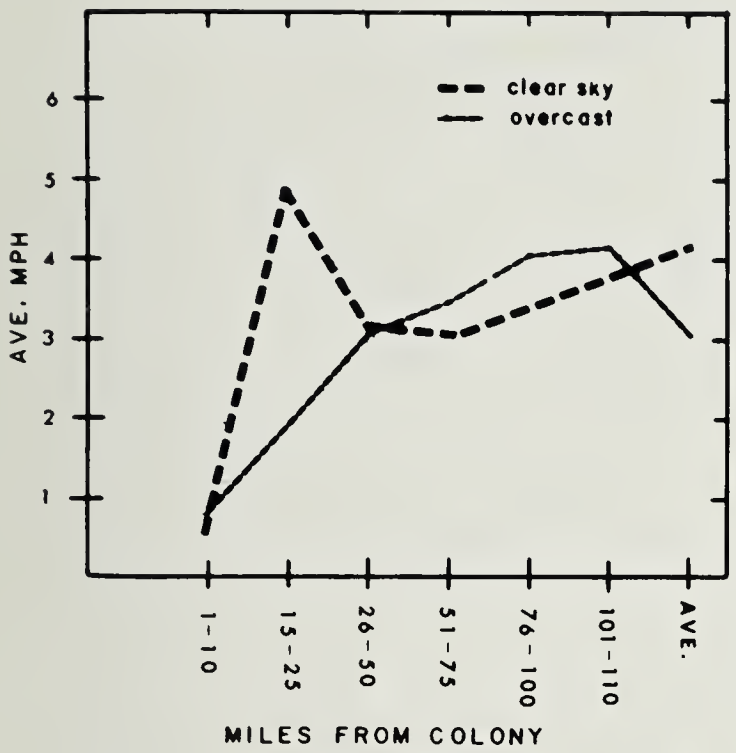
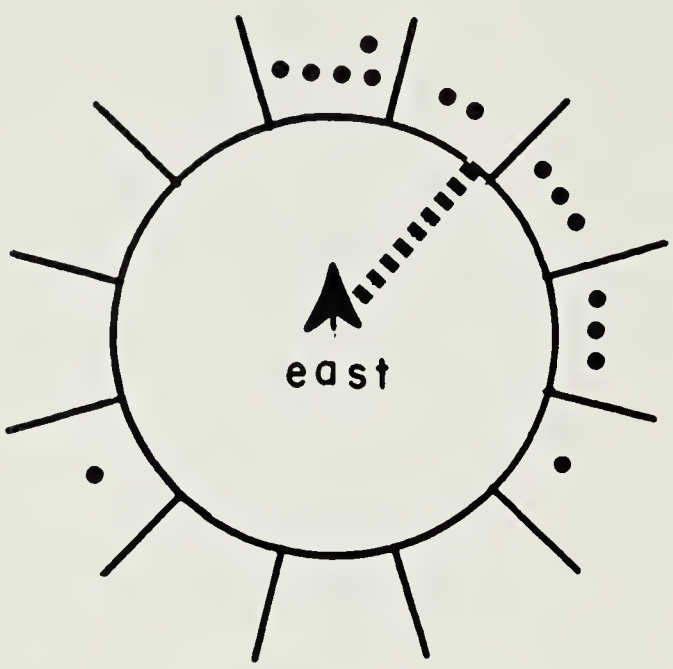


FIGURE 5. Average flight speeds of gulls released at various distances under clear and overcast skies (Skies A and D respectively).

titudes on overcast days).

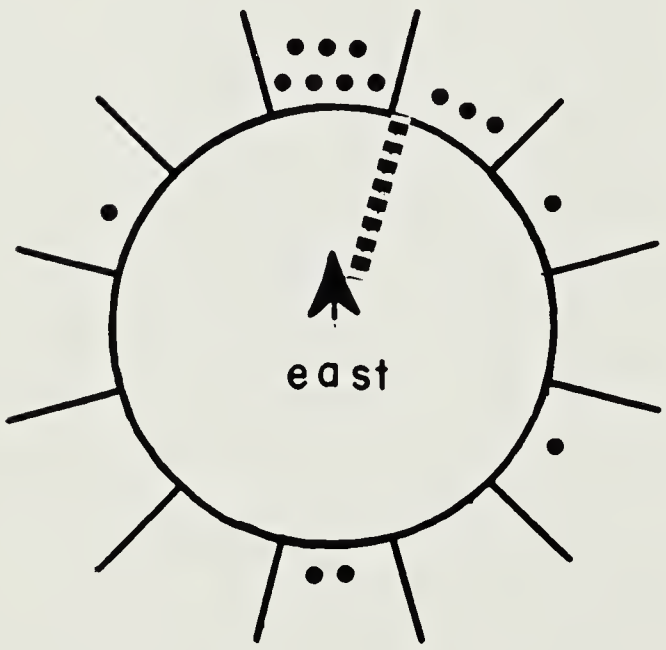
Some localities at which gulls were released (2-15 mile locations) were within the normal feeding range of the population and, in addition, high flying gulls were probably able to see Lake Huron. When birds were able to see large bodies of water, they usually headed toward them, regardless of the direction from home. Other behaviors also indicated that landmarks (*e. g.*, rivers, high hills) affected homing behavior (Southern, 1967a). Possibly gulls released under the four sky conditions at distances over 15 miles from home also responded to landmarks during trials and the range of performances I recorded was the result of individual differences in familiarity with the terrain or in homing ability. In either case, experiences acquired during one flight should modify the future responses of individuals during migration and possibly during homing trials. However, successful homers were not, in most instances, more efficient during later trials (Southern, 1969b). The reason for this may be that some gulls react less favorably to handling and the other treatment usually associated with the experiment and are, for that reason, more hesitant to return to the colony. Several radio-tracked gulls returned to within sight of the colony but failed to enter. This type of behavior could account for the apparent range of homing abilities the gulls exhibited. It is important to remember that birds are probably reacting to a variety of stimuli during homing experiments and that each of their responses need not be related to stimuli associated with displacement from their familiar territory. Furthermore, during migration and homing experiments, birds may not be motivated to perform rigorous flights during unfavorable conditions. Instead, initial departure may be in other than homeward directions and homeward progress may be slow, or absent, prior to improved conditions.

The usual measures of homing (orientation) ability relative to the presence of solar cues have been based on initial flight directions, departure directions, homing success rates, and/or comparative homing speeds. Each of these variables has been considered during this project and no evidence supports a hypothesis that solar clues are essential for orientation as performed by Ring-billed and Herring Gulls. I believe that orientation by these species is equally efficient during the presence, or absence of the sun. Proponents of a sun hypothesis may argue that the distances used during these trials were too short and that gulls were



SKY A

SKY B-C



SKY D

FIGURE 6. Departure headings of juvenile Ring-billed Gulls in relation to variations in sky conditions. Broken lines represent mean departure angles. Standard deviations: Sky A, 47.76; BC, 75.60; D, 53.60.

homing by means of landmark recognition. While I concur with the possibility of landmark orientation, my radio-tracking data and direct observations indicated that the gulls did not appear to recognize release localities that were more than 15 miles inland. In fact, inland displacement of lesser distances may have caused some disorientation.

The possibility remains that the sun, whenever visible, might be used to some extent in compass-orientation, thereby supplementing the method used at other times. In such instances, the sun would only pro-

vide a gross clue for aid in selection of a general heading.

Fluctuations in the earth's magnetic field was the only environmental factor that could be correlated with alterations in directional preferences of young Ring-billed Gulls. Chicks showed reduced preferences for southeast, east, and south headings with increasing levels of magnetic disturbance (based on K-values of magnetic storm activity obtained from the Coast and Geodetic Survey's Fredricksburg Observatory). The existing data (Southern, in press) are still inconclusive but suggest that factors other than celestial cues or geographical features are involved in the selection of initial migratory headings by young gulls. Further studies are being conducted. If young gulls possess an inherited ability to select appropriate headings for initial migration, the adults should also; however, their basic response pattern may be modified by experiences gained during migrations, feeding flights, and other activities. As a result, adults fail to show similar tendencies during homing experiments.

Summary

Of 429 adult and subadult Ring-billed and Herring Gulls used in homing trials, 41 of the Herring Gulls were radio-tracked. Homing behavior was contrasted with existing sky conditions. No evidence was obtained that supported the use of the sun or stars as orientational cues by these species. Juvenile Ring-billed Gulls showed preferences for southeast and east headings when tested during homing trials, suggesting that members of these species have an innate ability to select courses suitable for reaching the winter range. Such behavior is modified in adults as a result of experiences gained during migration.

Acknowledgments

This research was supported by grants from the Frank M. Chapman Memorial Fund (American Museum of Natural History) and The Society of Sigma Xi. Jerrold H. Zar prepared the computer program that was run on an IBM 360/40 in the NIU Computer Center.

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NEW TAXA IN *XIMENIA* (Olacaceae)

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ABSTRACT. — *Ximenia americana* Linnaeus var. *argentinensis* of Argentina, Paraguay and Bolivia and *Ximenia parviflora* Bentham var. *glauca* of Mexico are newly described. *Ximenia coriacea* Engler var. *intermedia* Chodat & Hassler, of Paraguay and Brazil, is elevated to species rank. The new varieties are illustrated.

Ximenia, a member of the arborescent dicotyledonous family Olacaceae, is composed of eight species (totaling twelve taxa) of tropical and subtropical, thorny root-parasites. The two new varieties and one new combination proposed below are the result of a study of specimens from thirty-eight herbaria (DeFilipps, 1968).

Infraspecific categories in *Ximenia* are customarily ranked as varieties, rather than subspecies, and this precedent is followed herein. All collections cited below have been examined by the writer; herbarium abbreviations are those of Lanjouw & Stafleu (1964).

The most widespread species in the genus is *Ximenia americana* Linnaeus, which has differentiated into three well-marked varieties. The typical element of this species, var. *americana*, is found in Florida (where it is known as tallow wood or hog plum), the West Indies, Mexico, Central and South America. In the Old World it is distributed in India, Cambodia, China, numerous island groups throughout the Pacific Ocean, and on the west coast and inland areas of Africa. In Africa this species is also represented by var. *microphylla* Welwitsch ex Oliver, which is restricted to the south-central part of the continent. Variety *microphylla* has glaucous, generally smaller leaves and usually more pronounced thorns than in var. *americana*, perhaps a reflection of the more xeric environmental conditions under which it grows. Examination of South American collections of this species has shown that a previously unrecognized variety occurs in Argentina, Paraguay and Bolivia. This taxon has glaucous, smaller leaves and is very thorny, as in its African counterpart (var. *microphylla*), and also possibly may have originally retained these characters in order to successfully occupy a drier habitat than var. *americana*.

This new element, described below as var. *argentinensis*, is separable from the other varieties as follows:

1. Leaves not glaucous; inflorescence an umbel, subumbel or cyme; sepals ciliate .... var. *americana*
1. Leaves predominantly glaucous; inflorescence a subumbel, or flowers solitary; sepals ciliate or eciliate.
   2. Petals 4.5-6.0 mm long; sepals ciliate or eciliate; style to 1.3

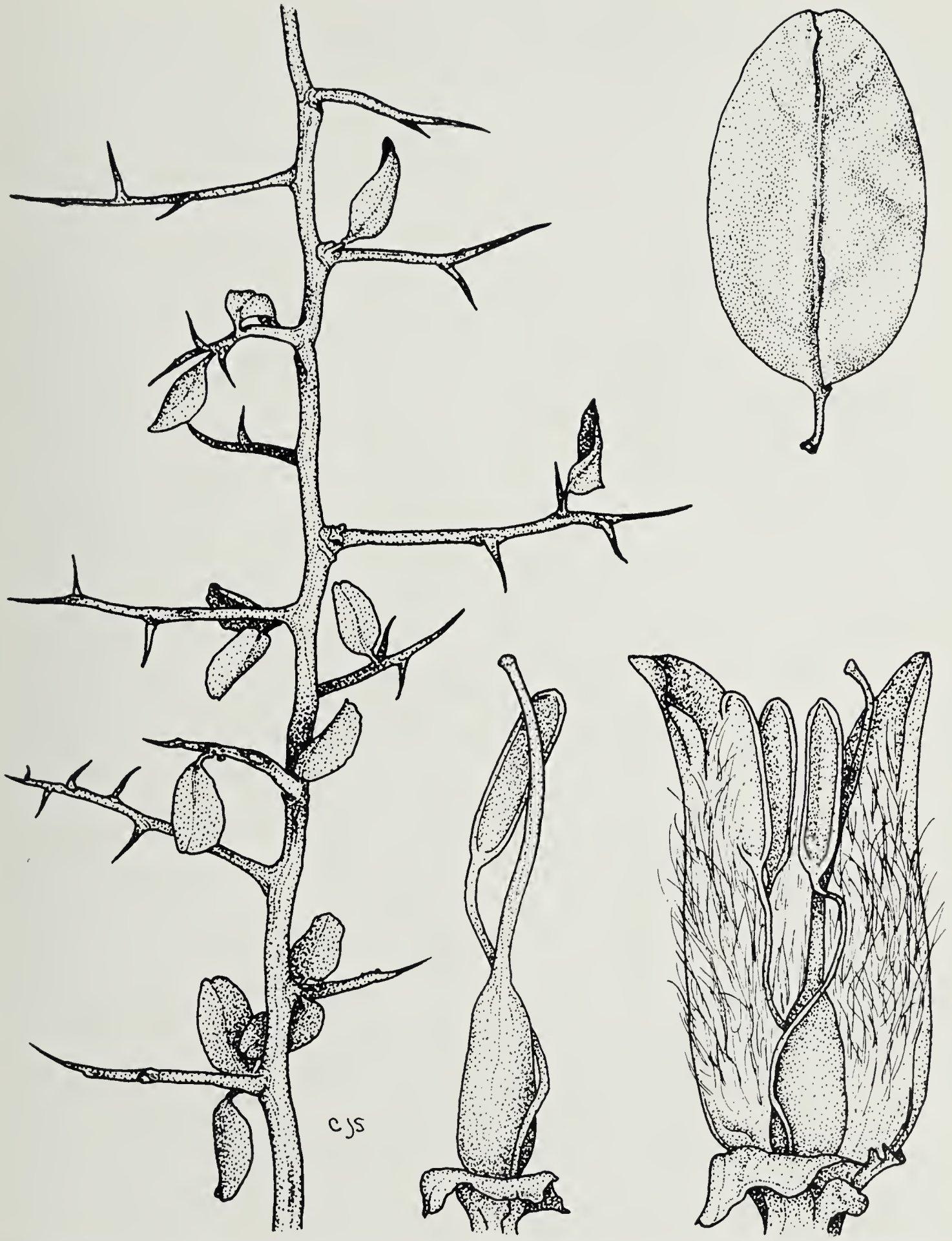

FIGURE 1. *Ximenia americana* Linnaeus var. *argentinensis* DeFilipps. Branch: XI, *Cocucci, DiFulvio & Artico 370* (SIU). Leaf: X4, *Cocucci, DiFulvio & Artico 370* (SIU). Flowers: X7, *Hunziker 8504* (SIU). Drawn by C. Seliger.

mm long . . . . var. *microphylla*

2. Petals 6.4-11.0 mm long; sepals eciliate; style 4.2-7.0 mm long . . . . . var. *argentinensis*

1. *Ximenia americana* Linnaeus var. *argentinensis* DeFilipps, var. nov. (Figure 1).

Glabri saepe glauci arbores vel frutices; inflorescentiae subumbellae

vel flores solitarii; sepala eciliata, petala (6.4-) 7.0-11.0 mm longa; styli (4.2-) 4.5-7.0 mm longi; differt a varietate americana et varietate microphylla longis stylis.

Glabrous, predominantly glaucous shrubs or trees 1-8 m; thorns to 5 cm long; lamine elliptic or orbiculate, retuse or obtuse, emucronulate or mucronulate, 1.3-3.0 (-4.1) cm long, 0.8-1.6 (-3.0) cm wide, membranous, often glaucous, petioles 2-4 (-6) mm long; inflorescences subumbels of 2-3 flowers, or flowers solitary, peduncles to 8 mm long, pedicels to 8 mm long, 2-bracteolate at 3 mm from base when solitary, or ebracteolate; flowers functionally bisexual, sepals (3-) 4 (-5), 1-2 (-4) mm long, eciliate, petals 4 (-5), (6.4-) 7-11 mm long, inner surfaces white-bearded to within 2 mm of apex, white or whitish-yellow; stamens 8, filaments 3.5-4.5 (-6.0) mm long, anthers (3.2-) 4.0-4.5 mm long; ovary lanceoloid, 2.5-3.0 (-4.0) mm long, style (4.2-) 4.5-7.0 mm long; drupe broadly ellipsoid, 1.6-2.3 cm long, 1.1-2.0 cm in diameter; seed ellipsoid, 1.4 cm long, 1 cm in diameter.

TYPE: ARGENTINA: Catamarca: Andalgala: Andalgala, 1910. *Jorgensen 1140.* (HOLOTYPE: US 921775; ISOTYPES: GH, MO, UC).

This variety occurs in monte vegetation in lowlands and mountainous areas in Argentina and in the Chaco of Paraguay and Bolivia. In Argentina the plants are locally called "pata" (*Luna 350; Venturi 282, 7208; Schreiter 4460*) or "albaricoque de campo" (*Hicken s.n.*).

Additional specimens examined:

ARGENTINA: BUENOS AIRES: Without locality, *Bacle s.n.* (G). CATAMARCA: El Alto: Balcogna, *Venturi 7208* (F), La Paz: Palo Cruz las Cefas, *Brizuela 396* (LIL), San Lorenzo, *Brizuela 94* (LIL), San Jose, *Brizuela 45* (LIL). CORDOBA: Cordoba, *Stuckert 3759* (G), Punilla: Los Cocos, *Stuckert 19628* (G), Sobremonte: Cerro Colorado, *Hunziker 8504* (SIU), Tulumba: Dean Funes a Sauce Punco, *Meyer 13237* (LIL). CORRIENTES: Empedrado: Arroyo Peguaho, *Pedersen 1182* (BR, S, U, US). ENTRE RIOS: Parana: La Toma, *Burkart, Troncoso & Bacigalupo 23645* (SI). JUJUY: Jujuy: 40 km S of Jujuy, *Eyerdam & Beetle 22430* (G,GH,UC), San Pedro: San Pedro de Jujuy, *Venturi 10537* (SI). LA RIOJA: General Belgrano: Olta, *Cocucci, DiFulvio & Artico 370* (SIU), General Roca: Chepu, *Cuezzo 993* (S), Independencia: Guayapas, *Hayward & Legname 3203* (LIL). MENDOZA: Maipu: Rio Mendoza, *Bartlett 19367* (GH,SI, US). SALTA: Anta: 5 km from Apolinario Saravia, *Morello, Legname & Cuezzo 905* (LIL), Campo Santo: Yaquiasme, *Meyer s.n.* (LIL), Chicoana: El Carril, *Burkart 17576* (SI), La Candelaria: Unquillo, *Schreiter 6640* (LIL,U), Oran: Rio Pedros, *Rodriguez 1105* (SI). SAN JUAN: Ullun: Rio San Juan, *Rodrigo 2820* (LIL). SAN LUIS: *Hicken s.n.* (SI). SANTA FE: Malabrigo, *Parodi 11267* (F,GH). SANTIAGO DEL ESTERO: Copo: Matoque, *Luna 338* (BR,LIL), Jiminez: El Charco, *Schreiter 5949* (U), *Venturi 9696* (A,MO,US), La Banda: Desmonte, *Luna 1282* (LIL), Ojo de Agua: Ojo de Agua, *Baleguo 1378* (LIL), Pellegrini: Algarrobal Viejo, *Luna 350* (LIL), El Tigre, *Luna 18* (LIL,

MO), Sarmiento: Garza, *Huidobro 3098* (LIL). TUCUMAN: Burruyacu: Burruyacu, *Stuckert 9295* (G), Trancas: Vipos, *Vervoorst 3350* (LIL), Tapia, *Venturi 1058* (US).

PARAGUAY: Alto Paraguay, Chaco, *Fiebrig 1360* (G,S,Z), Gran Chaco, Loma Clavel, *Hassler 2568* (BM,G).

BOLIVIA: Santa Cruz: Boyuibe, *Troll 436* (B).

2. *Ximenia parviflora* Bentham var. *glauca* DeFilipps, var. nov. (Figure 2).

   Differt haec varietate a varietate parviflora glaucis caulibus et foliis, foliis obovatis vel orbiculatis.

Glaucous, glabrous or puberulent, divaricately branched shrubs 0.5-2.5 m; thorns to 1.5 cm long; laminae obovate or orbiculate, retuse or obtuse, emucronulate or with mucro to 0.4 mm long, 1.2-2.0 cm long, 0.9-1.5 cm wide, subcoriaceous, glabrous or puberulent, petioles 2.5-4.0 mm long; inflorescences subumbels of 2-3 flowers, or flowers solitary, peduncles 1.5 mm long, pedicels of solitary flowers 3.5-7.5 mm long, 2-4-bracteolate at 0.5-2.0 mm from base; flowers functionally bisexual or unisexual, sepals 4, 1 mm long, sparsely ciliate, petals 4, 5.5-6.0 mm long, inner surfaces white-bearded to within 1.5-2.0 mm of apex, maroon; stamens 8, filaments 1.8-3.0 mm long, anthers 1.8-2.8 mm long; functional ovary lanceoloid, (2.2-) 2.6-3.0 mm long, style (0.3-) 0.5-1.5 mm long; ovary of functionally male flowers lanceoloid, 2.0-2.5 mm long, style absent; drupe globoid, 1.3 cm long, 1.1 cm in diameter; seed globoid, 1.3 cm long, 1.1 cm in diameter.

TYPE: MEXICO: Baja California Sur: Sierra de la Giganta, arroyo in the vicinity of Rancho Naucajoa, W of Llanos de San Juan, with *Olneya tesota, Cercidium microphyllum, Lysiloma candida, Bursera epinnata, Lemaireocereus thurberi,* and *Pachycereus pringlei,* altitude ca. 120-200m, May 31, 1963. *Carter & Reese 4534.* (HOLOTYPE: UC; ISOTYPE: SIU).

Ximenia parviflora var. *parviflora,* the typical element of this species, occurs on mainland Mexico in the States of Chiapas, Guanajuato, Hidalgo, Jalisco, Michoacan, Nayarit, San Luis Potosi and Sinaloa. It is characterized by elliptic, oblong or spatulate leaves which are not glaucous. In contrast to this, the stems and leaves of var. *glauca* are conspicuously glaucous, and the leaves are obovate or orbiculate. These features, in addition to its geographical confinement to the peninsula of Baja California, serve to separate var. *glauca* from the mainland variety.

Variety *glauca* appears to be limited to the inland northeastern flanks of the Sierra de la Giganta (essentially glabrous specimens) and the adjacent coastal arroyos of the Gulf of California (densely puberulent specimens, as *Carter & Ferris 4702* and *Johnston 3718*). When present, pubescence is usually confined to stems and leaves, however *Johnston 3718* has densely puberulent stems, leaves, petioles, pedicels and calyces. This condition suggests a relationship to *Ximenia pubescens* Standley, an incompletely-known species of Oaxaca, Mexico, although the latter has non-glaucous foliage and the

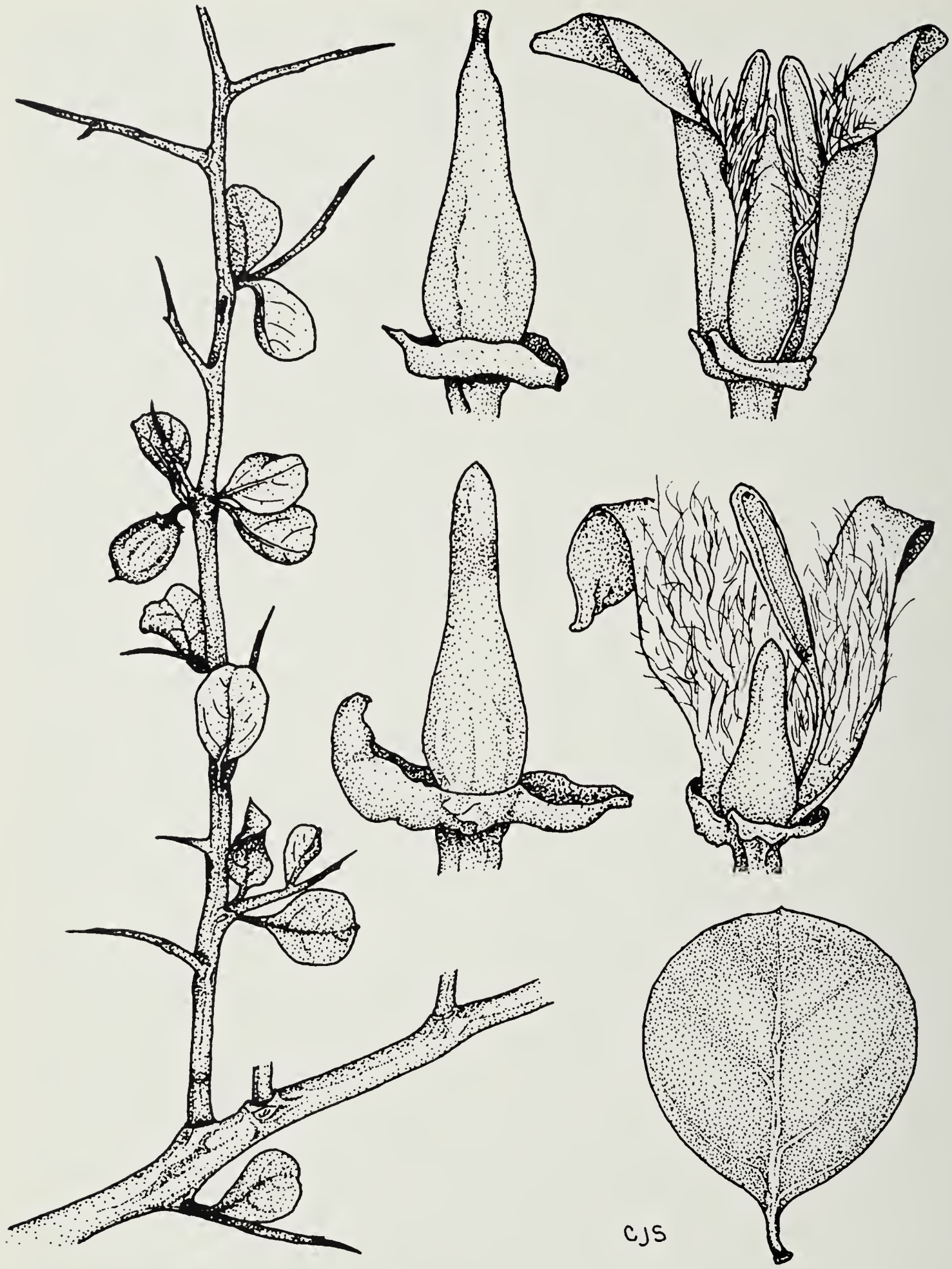

FIGURE 2. *Ximenia parviflora* Bentham var. *glauca* DeFilipps. Branch: X1, *Carter & Reese 4532* (SIU). Leaf: X2, *Carter & Reese 4532* (SIU). Functionally bisexual flowers at upper right: flower (at right) X8, pistil (at left) X12, *Carter & Reese 4534* (SIU, isotype). Functionally male flowers at center right: flower (at right) X10, pistil (at left) X16, *Carter & Reese 4532* (SIU). Drawn by C. Seliger.

exterior surfaces of the petals are also puberulent.

The local name recorded for var. *glauca* is "bachata" (*Carter 4469*).

Additional specimens examined:

MEXICO: Baja California Sur: Sierra de la Giganta, Cuesta de Naucajoa, W of Llanos de San Juan, *Carter & Reese 4532* (SIU,UC), *Carter 4469* (UC), Arroyo de Gua, NW of Loreto, *Carter & Ferris 3973* (UC), Mouth of arroyo entering Gulf S of Punta San Basilio, *Carter & Ferris 4702* (UC), San Nicholas Bay, *Johnston 3718* (CAS 84631, UC).

3. *Ximenia intermedia* (Chodat & Hassler) DeFilipps, comb. nov. and stat. nov.

Ximenia coriacea Engler var. *intermedia* Chodat & Hassler, Bull. Herb. Boiss. Ser. 2. 4: 64. 1903.

Shrubs 1-4 m; thorns 1.2-2.3 cm long; laminae elliptic to ovate, obtuse or retuse, with mucro to 0.5 mm long, 3.2-5.5 cm long, 1.9-2.6 (-3.6) cm wide, membranous to coriaceous, glabrous, petioles 3.0-4.5 mm long, glabrous to puberulent; inflorescences subumbels or umbels of 2-4 flowers, peduncles 1.5-4.5 mm long, puberulent, pedicels 2-3 mm long, glabrous to puberulent; flowers functionally bisexual, sepals 4 (-5), 0.3-0.6 mm long, glabrous to puberulent, ciliate at apex, petals 4 (-5), 4.5-6.0 mm long, inner surfaces white-bearded to within 0.5-2.0 mm of apex, ciliate, white or yellow-green; stamens 8, filaments 2.0-4.2 mm long, anthers 1.7-2.5 mm long; ovary lanceoloid, 1.8-3.0 mm long, style 1.4-1.5 (-2.0) mm long; drupe ellipsoid, 1.5 cm long, 1.3 cm in diameter, seed 1.2-1.3 cm long, 0.9-1.0 cm in diameter.

TYPE: PARAGUAY: In regione cursus superioris fluminis Apa, Jan. 1901-1902. *Hassler 8408*. (HOLOTYPE: G; ISOTYPES: A,BM,F, MO,UC).

The 2- to 4-flowered inflorescences, puberulent peduncles, and often puberulent pedicels and sepals characterize this species. The short style indicates a relationship to glabrous *Ximenia coriacea* Engler of the States of Minas Gerais and Bahia, Brazil, which has solitary flowers and styles 1.5-1.7 mm long.

This species occurs in the cerrados of Paraguay and Brazil. In Brazil the plants are locally called "limão-sinho" (*Macedo 1180*).

Additional specimens examined:

PARAGUAY: Apa River, *Hassler 8404* (S), Sierra de Amambay, Esperanza, *Hassler 10613* (A,B,G), *10613a* (A,G,MICH,MO,S,SI,UC, US).

BRAZIL: MINAS GERAIS: Ituiutaba, *Macedo 1180* (S,US), *2584* (S), Without locality, *Claussen s.n.* (G). BRASILIA D.F.: *Barroro 650* (SIU). MATO GROSSO: Pantanal de Rio Negro, Fazenda São Sebastião, *Castellanos & Strang 22523* (R).

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A RECONSTRUCTION OF THE FROND OF *KALYMMA*

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ABSTRACT. — A reconstruction of the frond of *Kalymma* is proposed. It consists of a basal petiole that bifurcates into two equal parts. Each of the petiolar divisions bears alternately arranged pinnae. The reconstruction is compared to several compression fronds.

The New Albany shale of Indiana and Kentucky contains a large flora of Upper Devonian and Lower Mississippian age. The flora consists of petrified fragments of lycopods, sphenopsids, ferns, progymnosperms, and gymnosperms. The fragmentary remains create taxonomic problems. Minor morphological differences between specimens were deemed sufficient to establish new genera and species. This taxonomic "splitting" was the only path open to the early workers (i.e. Scott and Jeffrey, 1914; Read, 1936a, 1936b, 1937; Read and Campbell, 1939).

As new specimens were collected and studied, some of the differences between genera were shown to be more imaginary than real by the discovery of specimens combining generic features and bridging the structural differences presumed to be present in the various genera. Examples of genera shown to be identical are: *Calamopteris* and *Kalymma* (Read, 1937); *Mesoneuron simplex* and *Stenokoleos* (Beck, 1960), *Arnoldella* and *Kalymma* (Sebby and Matten, 1968, 1969).

Probably the most common plant in the flora is *Kalymma*. This is a form genus representing petiolar and other frond axes of members of the family Calamopityaceae, an artificial assemblage of possible gymnosperms and progymnosperms. There are five species represented: *K. lirata, K. auriculata, K. resinosa, K. hippocrepis,* and *K. minuta.* Characters used to separate the species include, distribution of the vascular strands, shape of vascular strands, degree of tangential expansion and fusion between vascular strands, and presence of "secretory" sacs or canals. Seward (1917) believed that a single *Kalymma* species could represent part of the petiole of several stem species of *Calamopitys.* It is our opinion that several species of *Kalymma* represent different structural conditions present at various levels in the frond of *Calamopitys.* Previous work suggesting conspecificity of some of the species of *Kalymma* has implied that these forms may represent various levels in the frond. In this respect, Read (1937) commented on the similarity between *Kalymma auriculata* and *Kalymma hippocrepis,* and stated that *Kalymma grandis* and *Kalymma striata* are probably conspecific and both are very close to *Kalymma lirata.*

Since the criteria for separating the various species of *Kalymma* are relatively minor, one cannot be sure

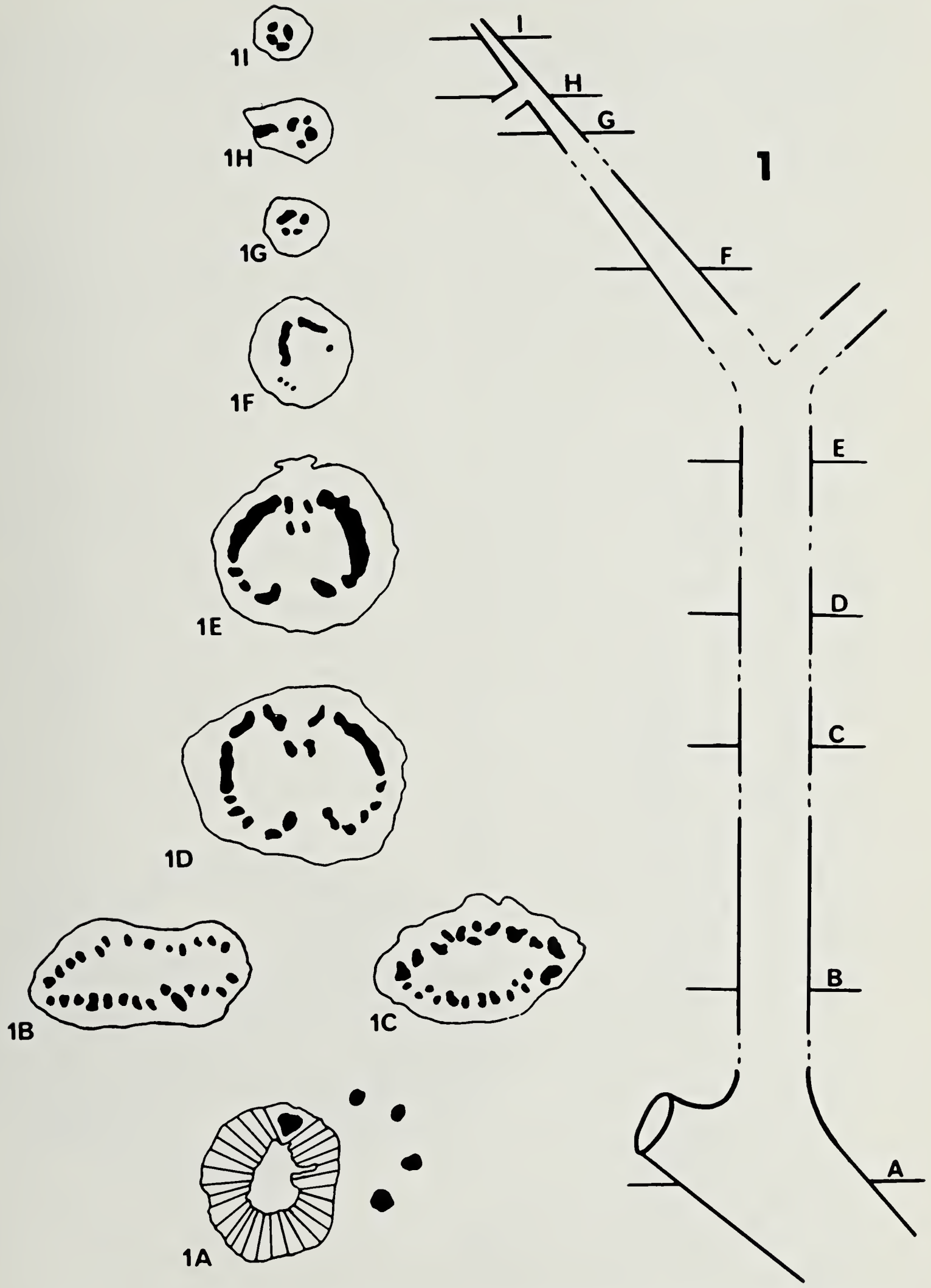

FIGURE 1. Hypothetical reconstruction of the frond of *Calamopitys* based upon numerous cross sections of several species of *Kalymma*: 1A - leaf base of *Calamopitys americana* (Read, 1937, plate 16, fig. 1); 1B - *Kalymma grandis* (Unger, 1856, plate 1, fig. 6); 1C - *Kalymma debilis* (Scott and Jeffrey, 1914, text fig. 2); 1D - *Kalymma lirata* (Read, 1937, plate 23, fig. 2); 1E - *Kalymma hippocrepis* (Scott and Jeffrey, 1914, text fig. 1); 1F - *Kalymma auriculata* (Read, 1936, text fig. 5); 1G, 1H, 1I - *Kalymma* sp.

of just how many of these features are indicative of the isolated axes present in the fossil record having come from several different plants. In addition, we wish to emphasize that many of the structural differences found among the specimens are due to varying structural patterns within a single frond. Examination of all of the species of *Kalymma* utilizing the numerous specimens now available enables us to present a generalized or model reconstruction of the frond (Fig. 1). This reconstruction may serve as a guide, to be modified as new specimens are found.

In *Calamopitys,* the vascular supply of the leaf originates as a single strand that divides several times to produce a polydesmic leaf base (Read, 1937). The base of the attached petiole has nine bundles in *Calamopitys saturnii* (Scott, 1923, p. 119) and three bundles in *Calamopitys annularis* (Scott, 1923, p. 117). A detached specimen of *K. lirata* has twenty bundles. Other large detached petioles show twenty-seven bundles in *K. grandis* (Unger, 1856, plate 1, fig. 6; Fig. 1-B) and twenty-six bundles in *K. debilis* (Scott and Jeffrey, 1914, text fig. 2; Fig. 1-C). It is interesting to note that Read (1937) thought these species (*K. grandis* and *K. debilis*) to be identical to *K. lirata.*

The low number of traces in the attached petiole base and the high number in the detached petioles indicate that the vascular bundles in the petiole base apparently continued to divide for a distance into the petiole forming a closed cylinder. Since no petioles have been found with a large diameter and few vascular bundles, transition from the petiole base with a few bundles to detached petioles with many bundles probably occurred within a relatively short distance.

We believe that the petiole dichotomized an unknown distance from the stem. Below the dichotomy, the cylinder of vascular bundles was invaginated adaxially and abaxially. Illustrative of this stage is *K. lirata* (Read, 1937, plate 24, fig. 1; Fig. 1-D). This was accompanied in some fronds by lateral fusion and tangential expansion of the bundles as seen in *K. lirata* (Read, 1937, plate 23, fig. 1,2). Further fusion and tangential expansion can be seen in *K. hippocrepis* (Scott and Jeffrey, 1914, text fig. 1; Fig. 1-E).

Above the bifurcation, each primary division of the petiole is characterized by having a C-shaped ring of bundles in transverse section. This is exemplified by *K. auriculata* in which some of the bundles remain fused forming tangentially expanded masses of vascular tissue while others form separate strands with a terete outline (Read, 1937, plate 23, fig. 6; Fig. 1-F).

The reconstruction of the petiole, below and above the dichotomy, is supported in two ways. First, the diameter of the axis of *K. auriculata* (Fig. 1-F) is approximately half that of *K. hippocrepis* (Fig. 1-E) and second, the configuration of the vascular tissue in *K. auriculata* is remarkably similar to that of a petiole of *K. hippocrepis* just above the primary division (if one extrapolates the division from the known specimens).

Knowledge of the anatomy of the axis of the primary division of the petiole is poor. In 1966, we collected

2

3

4

FIGURE 2. Serial transverse sections of *Kalymma* sp. showing arrangement of vascular strands.

FIGURE 3. Bioscope drawing of *Kalymma* sp. Note the ground mass parenchyma (GMP), the vaccular bundles (VB), and the sclerenchymatous outer cortex (SC).

FIGURE 4. Reconstruction of SIU K2.

a *Kalymma* specimen (SIU #K2) from the Junction City locality of the New Albany shale (Haroldsburg, Ky. Quadrangle, 84° 41′ W x 37° 35′ 45″ N). The specimen consisted of a main axis with a lateral appendage. The main axis was 4.5 to 5.3 mm wide and a cross section (Fig. 3) revealed four vascular strands. The small diameter and low number of vascular strands indicate that the specimen is either a distal portion of the primary division of the petiole or is a higher order of branching.

The vascular changes in the main axis can be followed by referring to the series of cross sections in Fig. 2 and to the reconstruction in Fig. 4. Section A in Fig. 2 represents the four-stranded vascular configuration below the lateral appendage. Of the four vascular strands, number 1 can be seen to divide twice (Fig. 2-I,J). The first division results in the formation of a trace (1a in Fig. 2-I) and a main axis strand (1b in Fig. 2-I). The second division (ie. the division of strand 1b) results in the formation of two main axis strands. In Fig. 2-I, strand 4 has become a trace. The two traces appear joined in the base of the lateral (Fig. 2-J). The four-strand configuration of the main axis is reconstituted by the second division of strand 1 (Fig. 2-L,M). A reconstruction of the vascular system is shown in Fig. 4. The dimensions have been slightly exaggerated for clarity. The vascular tissue in the lateral is a single V- or C-shaped strand in cross section (Fig. 2-K). The lateral appendage was perpendicular in its insertion on the main axis and was 1.3 mm wide. Read (1937) mentioned that he had examined several small specimens of *Kalymma* with lateral appendages and that these lateral appendages were supplied by several traces. However, he did not describe or illustrate the specimens.

In our reconstruction of the frond we assume that the number of vascular strands in the primary division of the petiole decreased in a distal direction. The production of lateral appendages may have contributed to the decrease. The lateral appendages were alternate in their attachment. As conceived, the hypothetical frond is compound, with a basal petiole bifurcating into two equal parts. Each of the primary divisions of the petiole bore alternately arranged pinnae.

Long (1964) suggested that the compression fronds *Diplothmema* and *Sphenopteridium* might be the frond of *Stenomyelon*. They could be the frond of *Calamopitys* as well. The foliage of *Diplothmema* and *Sphenopteridium* was of the *Sphenopteris* or *Rhodea* type (Kidston, 1923). *Sphenopteridium* differs from *Diplothmema* in that pinnae are produced below the dichotomy of the petiole.

Although no reconstruction for the frond of *Diichnia* (Read, 1936b) is provided, we suggest that *Kalymma resinosa* with its complete cylinder of vascular strands represents the basal portion of the frond. Some specimens of *K. resinosa* show the ring of vascular strands invaginated adaxially and abaxially in the region of a dichotomy. It seems likely that *Kalymma minuta* (Sebby and Mat-

ten, 1968, 1969) with its C-shaped stele represents a primary division of the petiole of the frond.

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PRIMARY PRODUCTIVITY IN AN ILLINOIS RIVER FLOOD PLAIN LAKE

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ABSTRACT. — Physico-chemical conditions and community metabolism in a shallow flood plain lake of the Illinois River were studied to determine the influence of the river on the lake. When the lake was inundated by the river, planktonic community structure and seasonal succession were disrupted resulting in minimal chlorophyll concentrations and assimilation numbers. Wind created turbidity in the lake appeared to be the major factor limiting primary production between periods of inundation.

Physico-chemical conditions and biota of flood plain lakes adjacent to the Illinois River have been extensively and intensively studied. None of these investigations, however, have reported quantitative studies of community metabolism. Shallow flood plain lakes of the Illinois River can be highly productive. These lakes receive nutrients, especially nitrates and phosphates, from the polluted Illinois River during periods of high flow. In the present study, community metabolism in a shallow flood plain lake was estimated by diel changes in oxygen concentration.

DESCRIPTION OF STUDY AREA

Worley Lake is a shallow flood plain lake of the Illinois River. It is located on the east bank of the river in Tazewell County approximately four miles south of Peoria, Illinois (Fig. 1). The lake covers an area of 156 hectares and has a maximum depth of one and one-half meters when at full capacity. Average depth is 0.7 m at full capacity. The bottom of the lake consists of soft flocculent material that has been deposited by the river during periods of inundation. Rooted vegetation and floating leaf vegetation is absent.

PROCEDURES

Community metabolism was estimated by methods described by Odum and Hoskin (1958) and Odum and Wilson (1962). Diurnals were conducted on clear days at stations located at opposite ends of the lake (Fig. 1). The distance between stations was approximately 1,200 m. Duplicate water samples were taken every two hours in daylight and every three hours at night at both stations and analyzed for oxygen by the Alsterberg (Azide) modified Winkler method (APHA, 1965).

Physico-chemical conditions were determined at weekly intervals during the summer and monthly intervals during other seasons except for January and February of 1968 when the lake was frozen over. Chlorophyll *a* concentration was determined by filtering 100 ml aliquots through

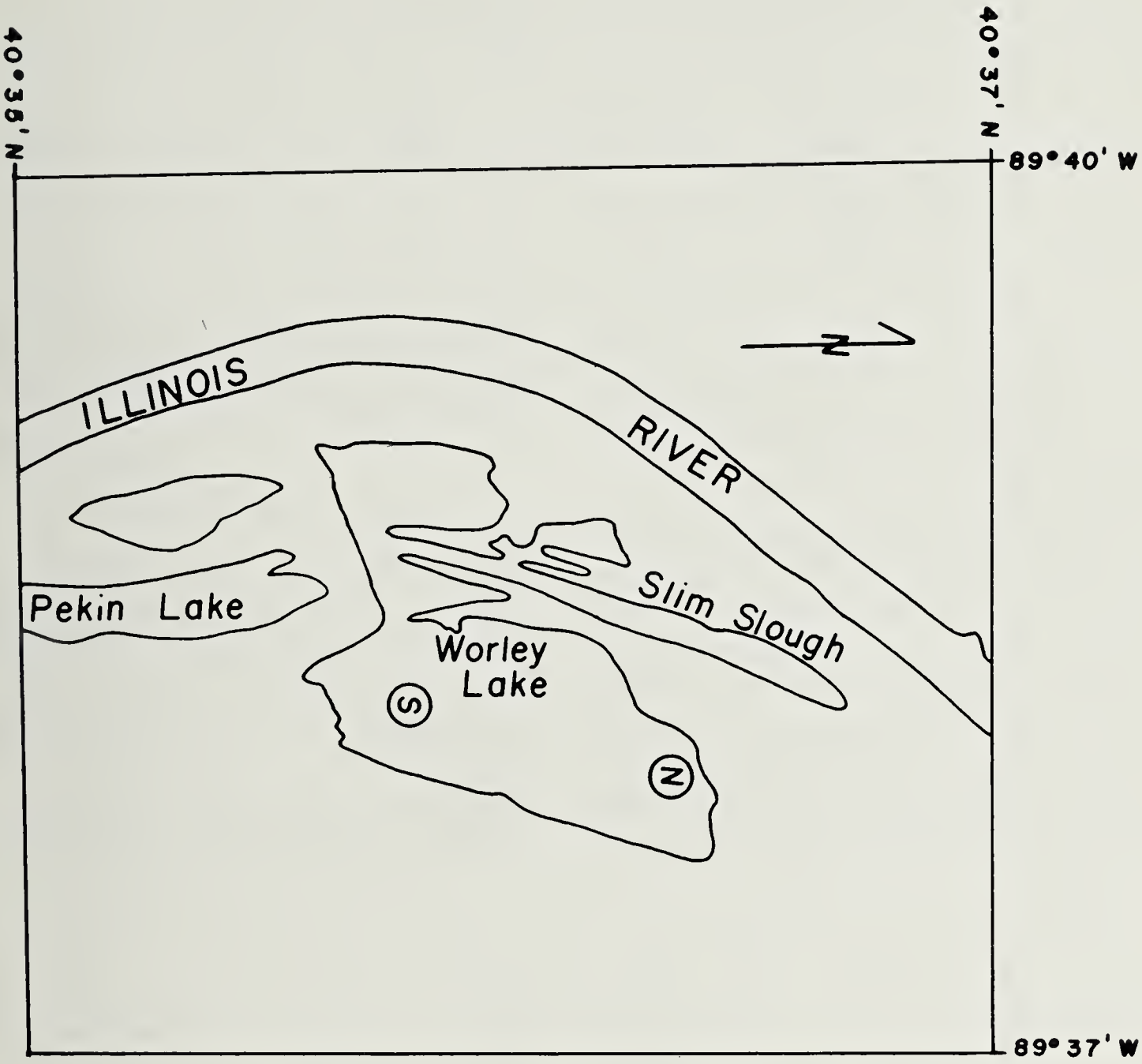

FIGURE 1. Map of Worley Lake and the adjacent Illinois River. S = South Station; N = North Station.

membrane filters of 0.45 micron pore size. The residues were extracted in 10 ml of 90% acetone and refrigerated in the dark at approximately 5 C for 24 hours. Upon removal, the samples were centrifuged and optical density of the liquid was determined with a Bausch and Lomb Spectronic 20 photoelectric colorimeter with a 2.54 cm cuvette at 430 and 665 millimicrons.

The methods of Odum, McConnell and Abbott (1958) were used to estimate chlorophyll *a* concentration where chlorophyll *a* in mg/liter (gr/m^3) of 90% acetone = 13.4 d_{665}. After correction for acetone and sample volume this equation became: Chlorophyll *a* in mg/liter = 1.34 d_{665}. Volumetric values were multiplied by depth of the euphotic zone to convert to an areal basis.

Water temperature and alkalinity were measured in the field according to standard methods. Turbidity was determined in the laboratory with a Bausch and Lomb Spectronic 20 photoelectric colorimeter with a 2.54 cm cuvette at d_{450}. The pH was measured in the field by means of a portable Sargent pH meter. Specific conductance was determined with a

TABLE 1. — Mean Annual Physico-Chemical Conditions.

Station	Turbidity (ppm)	Specific Conductance (umhos/cm)	ALKALINITY HCO_3 (ppm)	ALKALINITY CO_3 (ppm)	pH
South	95	501	138	5	8.4
North	102	491	130	9	8.6

YSI Model 31 conductivity bridge while the depth of the euphotic zone was measured with a G. M. Mfg. Instrument Corporation submarine photometer.

Suspended organic matter was determined by filtering 100 ml aliquots through membrane filters of 0.45 micron pore size. After filter and residue had been dried in an oven, they were cooled, weighed, and ashed at red heat in a muffle furnace. After cooling and weighing the ash, the weight of the ash and filter paper was subtracted from the dried weight to determine weight of suspended organic matter.

Results and Discussion

Physico-Chemical Conditions

Differences in mean annual physico-chemical conditions between stations were slight (Table 1). Turbidity varied from 25 to 275 ppm while pH varied fom 7.9 to 9.5. Specific conductance varied from 338 to 700 micromhos and was lowest

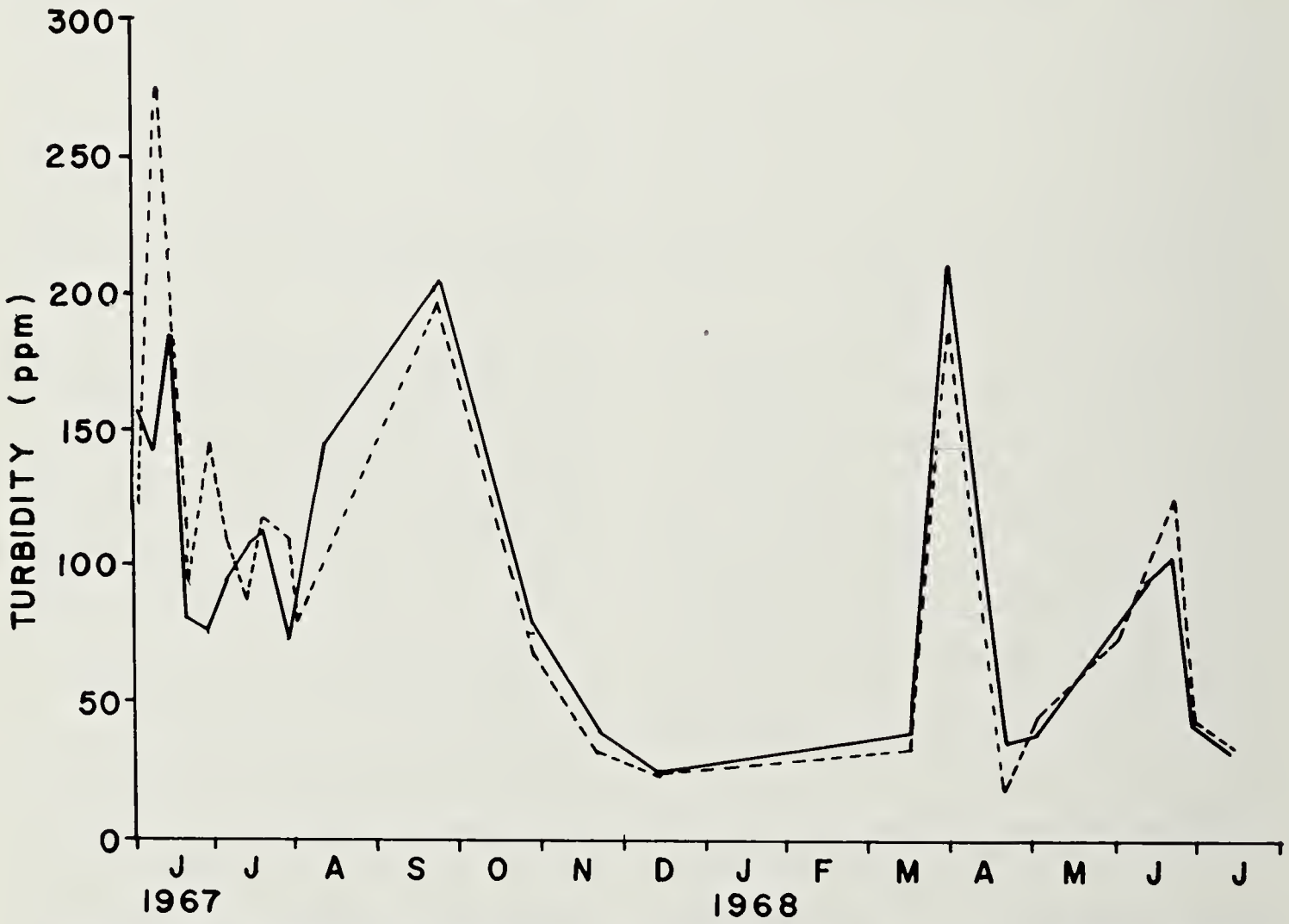

FIGURE 2. Turbidity conditions in Worley Lake. (——) = South Station; (- - -) = North Station.

after dilution by river water. The loss of water from the lake by evaporation at low flow periods of the lake apparently concentrated ions responsible for specific conductance measurements.

Water in Worley Lake is extremely turbid, especially when the water level is less than full capacity (Fig. 2). Turbidity ranged from 25 ppm to 205 ppm at the South Station and from 33 ppm to 275 ppm at the North Station.

The euphotic zone, considered to be that depth at which light penetration is 1% of surface intensity, varied considerably during the study but was generally deepest when the river was connected to the lake (Table 2). The deepest penetration of the euphotic zone into the lake occurred on 12 July 1968 while the river was in a prolonged flood stage. At that time the maximum water depth was 2.5 m. In their study of Lake Chautaugua, Jackson and Starrett (1959) found that turbidity tended to vary with wind velocity during low water periods if vegetation or ice cover was absent. A similar situation was noted in Worley Lake. When water level in the lake was low, wave action kept the turbidity high.

TABLE 2. — DEPTH OF EUPHOTIC ZONE IN METERS

Date	Station South	North
14 June 1967	.38	.41
22 June 1967	.55	.55
1 July 1967	.44	.55
23 July 1967	.65	.60
31 July 1967	.54	.54
14 Dec. 1967	1.00	1.00
16 Mar. 1968	.65	.78
29 Mar. 1968	.18	.16
19 Apr. 1968	.71	.71
2 May 1968	.51	.56
3 June 1968	.75	.67
12 June 1968	.46	.53
18 June 1968	.41	.41
21 June 1968	.38	.38
28 June 1968	.91	.91
12 July 1968	1.13	1.04
Mean	.60	.61

Chlorophyll and Suspended Organic Matter

Highest chlorophyll concentration observed was 0.64 mg/1 in March at the South Station and 0.75 mg/1 in April at the North Station (Fig. 3). According to Bartsch and Allum (1957), Odum et al. (1958) and Wright (1960) chlorophyll concentration is dependent upon the amount of available nutrients. Nutrient levels were apparently high at this time since large numbers of

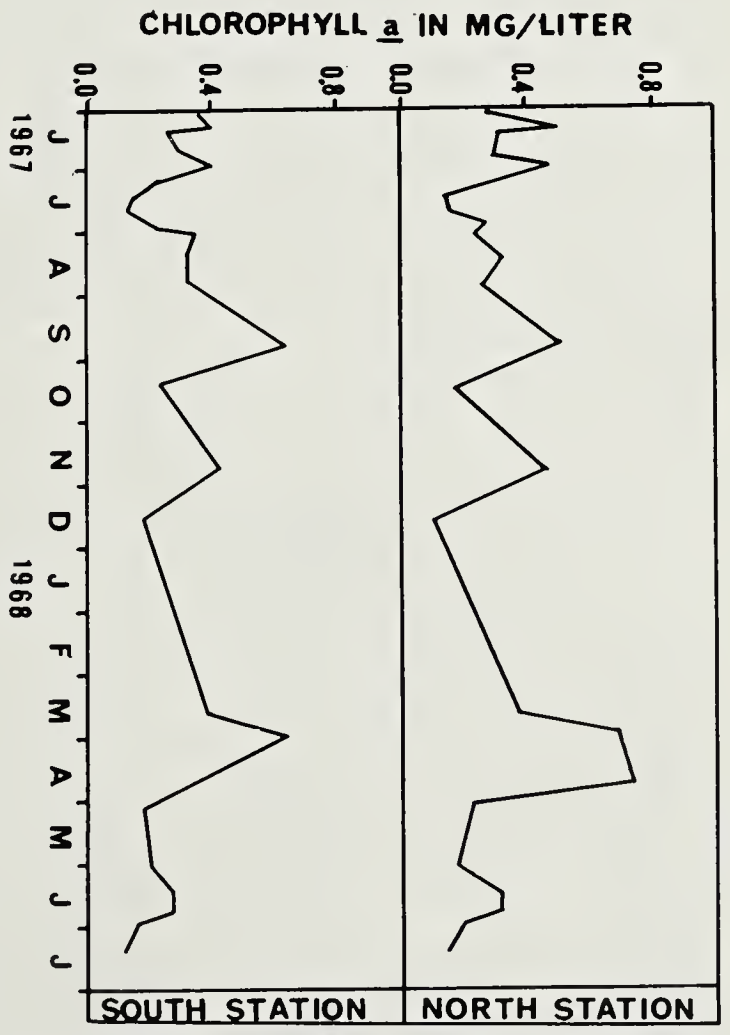

FIGURE 3. Chlorophyll *a* concentration in Worley Lake.

TABLE 3. — Summer Chlorophyll *a* and Suspended Organic Matter.

Station	Chlorophyll *a*				Suspended Organic Matter	
	Range (g/m^3)	Mean±SD (g/m^3)	Range (g/m^2)	Mean±SD (g/m^2)	Range (g/m^3)	Mean±SD (g/m^3)
South	0.12-0.63	0.30±0.12	0.08-0.20	0.16±0.05	22.1-60.0	33.4±10.1
North	0.15-0.52	0.28±0.16	0.08-0.26	0.16±0.05	16.2-65.1	38.0±16.4

dead fish were observed in and around the lake. These fish were apparently killed during late winter when the lake was ice covered. Suspended organic matter and chlorophyll *a* concentration for the summer are presented in Table 3. The large standard deviation for both suspended organic matter and chlorophyll *a* reflects the wide range of concentrations present in the lake.

Chlorophyll *a* concentrations in the Illinois River during summer are comparable to those found in Worley Lake during summer (Mathis, Root and Weiman, 1968). They reported that chlorophyll *a* concentration ranged from 0.03 to 0.50 mg/1. These concentrations are of the level found in ecosystems receiving large quantities of organic wastes.

Chlorophyll and Assimilation Numbers

The ratio of oxygen production to chlorophyll is referred to as the assimilation number (Odum et al., 1958). Assimilation numbers ranged from 0.2 to 10.7 g 0_2/g chlorophyll $m^{-2}hr^{-1}$ (Table 4). No consistent relationship between the assimilation number and chlorophyll concentration could be discerned in this study. In general, however, assimilation numbers were lowest during and immediately following pe-

TABLE 4. — Changes in the Relationship Between the Assimilation Number and Chlorophyll *a*

Date	South Station		North Station	
	Assimilation Number	Chlorophyll *a* (g/m^2)	Assimilation Number	Chlorophyll *a* (g/m^2)
6/14/67	0.3	.14	0.2	.17
6/25/67	7.1	.20	7.4	.23
6/30/67	2.2	.15	3.7	.18
7/22/67	2.8	.08	2.7	.09
7/31/67	2.8	.12	3.7	.15
12/14/67	0.9	.19	0.4	.12
3/29/68	0.7	.12	0.6	.11
5/4/68	1.7	.10	1.4	.13
6/3/68	2.5	.16	4.1	.13
6/18/68	2.6	.11	2.4	.12
6/21/68	2.4	.11	2.1	.11
7/12/68	10.6	.14	8.3	.15

riods of high river flow. The high assimilation number obtained in July, 1968, can be attributed to high productivity that occurred after the river had reached and maintained a stable flood stage for two weeks.

Other investigators have found a direct correlation between assimilation numbers and chlorophyll concentration. Copeland, Minter and Dorris (1964) in their study of oil refinery effluent holding ponds reported that assimilation numbers decreased as chlorophyll concentrations increased. Wright (1959) in his study of a natural community and Cooke (1967) in his study of laboratory microcosms reported similar observations. Late successional stages probably occur infrequently in flood plain lakes because frequent floods and high turbidity depress optimal growth conditions. Fluctuations in immature systems are more closely related to abiotic factors while more mature systems fluctuate because of changes in biotic factors (Margalef, 1963a; Hannan, 1967).

The ratio of plant pigment absorbancies (D_{430}/D_{460}) has been suggested by Margalef (1963a,b) as a means of determining succession. Margalef pointed out that the index increases as succession advances. Cooke (1967) reported an increase from an initial level of 2.3 to a maximum of 3.3 after which the index declined to 2.3 again. In Worley Lake, this index ranged from 2.76 to 3.38 at the South Station and from 2.67 to 3.71 at the North Station (Fig. 4). High indices in November and May suggest that the community was proceeding toward stabilization during periods of relative quiescence. The occurrence of

FIGURE 4. Variation in the Margalef pigment ratio in Worley Lake. (——) = South Station; (– – –) = North Station.

unstable conditions in December disrupted succession, however, and the index decreased. Repetitive disturbances in June kept the index lower than 3.00 at both stations. During July, 1968, however, the index increased when prolonged flood conditions kept the lake at a high but stable level. This suggests that succession was proceeding normally.

Community Metabolism

Primary production and community respiration are plotted for days with clear or nearly clear skies in figure 5. Gross photosynthesis ranged from 4.20 g 0_2m^{-2} day^{-1} in winter to 34.6 g $0_2m^{-2}day^{-1}$ in summer at the South Station. Community respiration varied between 4.4 g 0_2m^{-2} day^{-1} in winter and 23.6 g 0_2m^{-2} day^{-1} in summer.

At the North Station, gross photosynthesis exceeded community respiration slightly more often than at the South Station. Gross photosynthesis at the North Station ranged from 1.2 g 0_2m^{-2} day^{-1} in winter to 30.6 g $0_2m^{-2}day^{-1}$ in summer. Community respiration ranged from 1.5 g 0_2m^{-2} day^{-1} in winter to 23.6 g 0_2m^{-2} day^{-1} in summer. Similar ranges of community metabolism have been reported in other studies (Table 5).

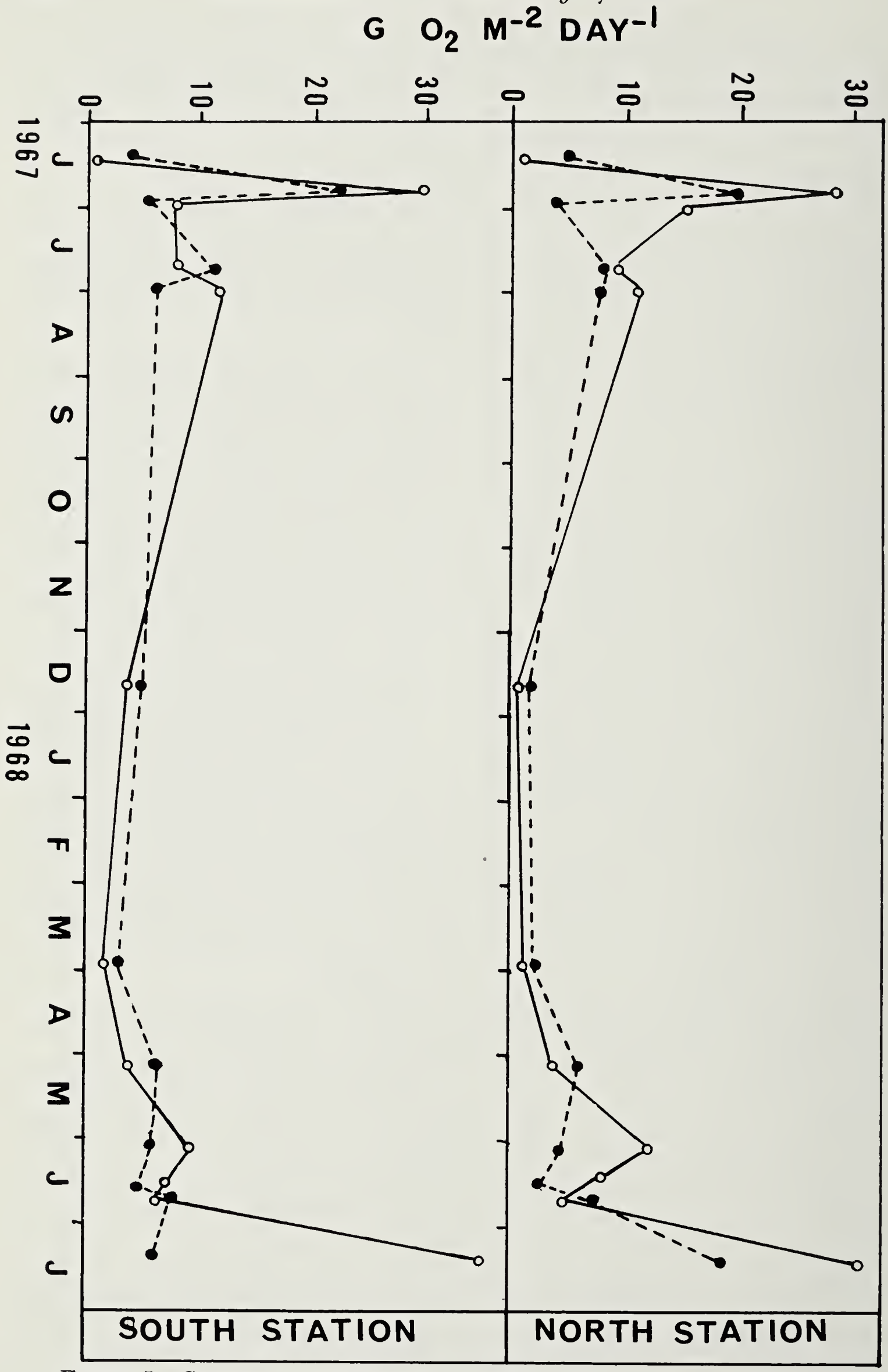

FIGURE 5. Community metabolism in Worley Lake. (——) = Gross Photosynthesis (– – –) = Community Respiration.

TABLE 5. — Community Metabolism in Selected Aquatic Communities Calculated from Diurnal Oxygen Curves

Source	Reference	g O_2/m^2/day Photosynthesis	Respiration
Unpolluted			
Blue River, Okla.	Duffer and Dorris (1966)	1.5-48.0	6.1-19.9
San Marcos River, Texas	Hannan (1967)	2.5-27.4	4.1-19.9
Polluted			
Skeleton Creek, Okla.	Baumgardner (1966)	2.8-60.4	4.9-81.8
Oil Refinery Effluent Holding Ponds, Okla.	Copeland and Dorris (1964)	0.0-29.2	2.1-50.5
Worley Lake, Illinois	Present Study	0.6-34.6	1.5-23.6

The variability exhibited by gross photosynthesis during summer can be attributed mainly to variations in turbidity and river level. Gross photosynthesis was highest during July, 1968, at the South Station when the river was at flood stage and connected to the lake. Water in the lake was extremely clear with the euphotic zone reaching to a depth of 1.13 m. Chlorophyll *a* concentrations during this particular diurnal, however, were not maximal. This suggests that light penetration into the water is the major factor limiting primary production in shallow lakes of this type, especially during spring and summer. Nutrient levels in the lake, especially phosphates and nitrates, are replenished each time the river overflows into the lake. Large quantities of sewage and industrial wastes are dumped into the Illinois River each day by cities and industries located within the drainage basin. As a result, the Illinois River has one of the highest phosphorus concentrations of any major waterway in the United States (Public Health Service Water Surveillance System, 1963).

The ratio of primary productivity to respiration (P/R ratio) ranged from 0.11 to 5.80 during the study (Fig. 6). This parameter also reflected unstable environmental conditions in the lake. When photosynthesis exceeds respiration (P/R ratio greater than unity) the community is autotrophic, and when photosynthesis is less than respiration (P/R ratio less than unity) the community is heterotrophic (Odum,

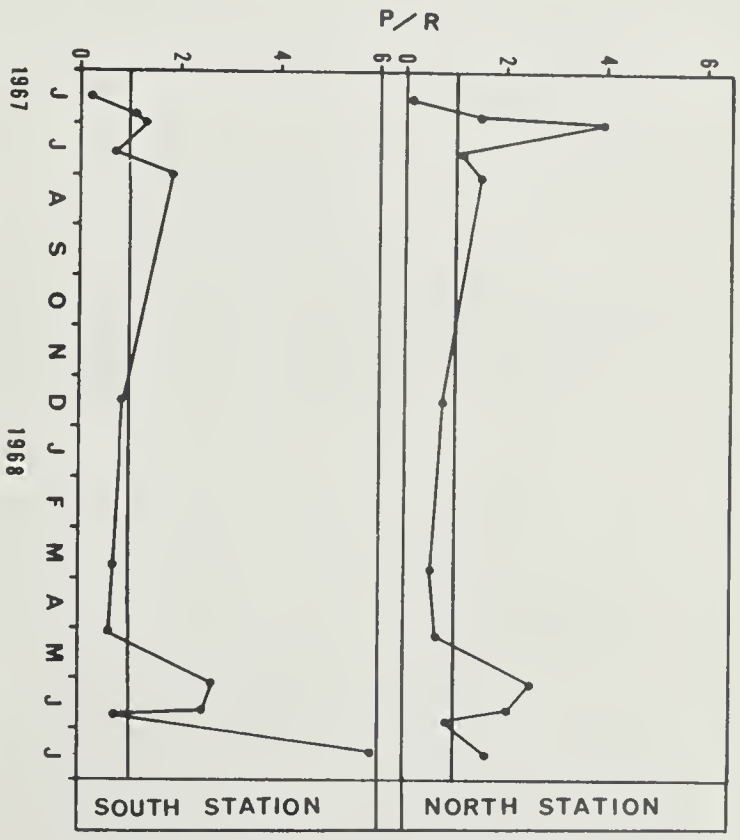

FIGURE 6. Photosynthesis/Respiration ratios of community metabolism in Worley Lake.

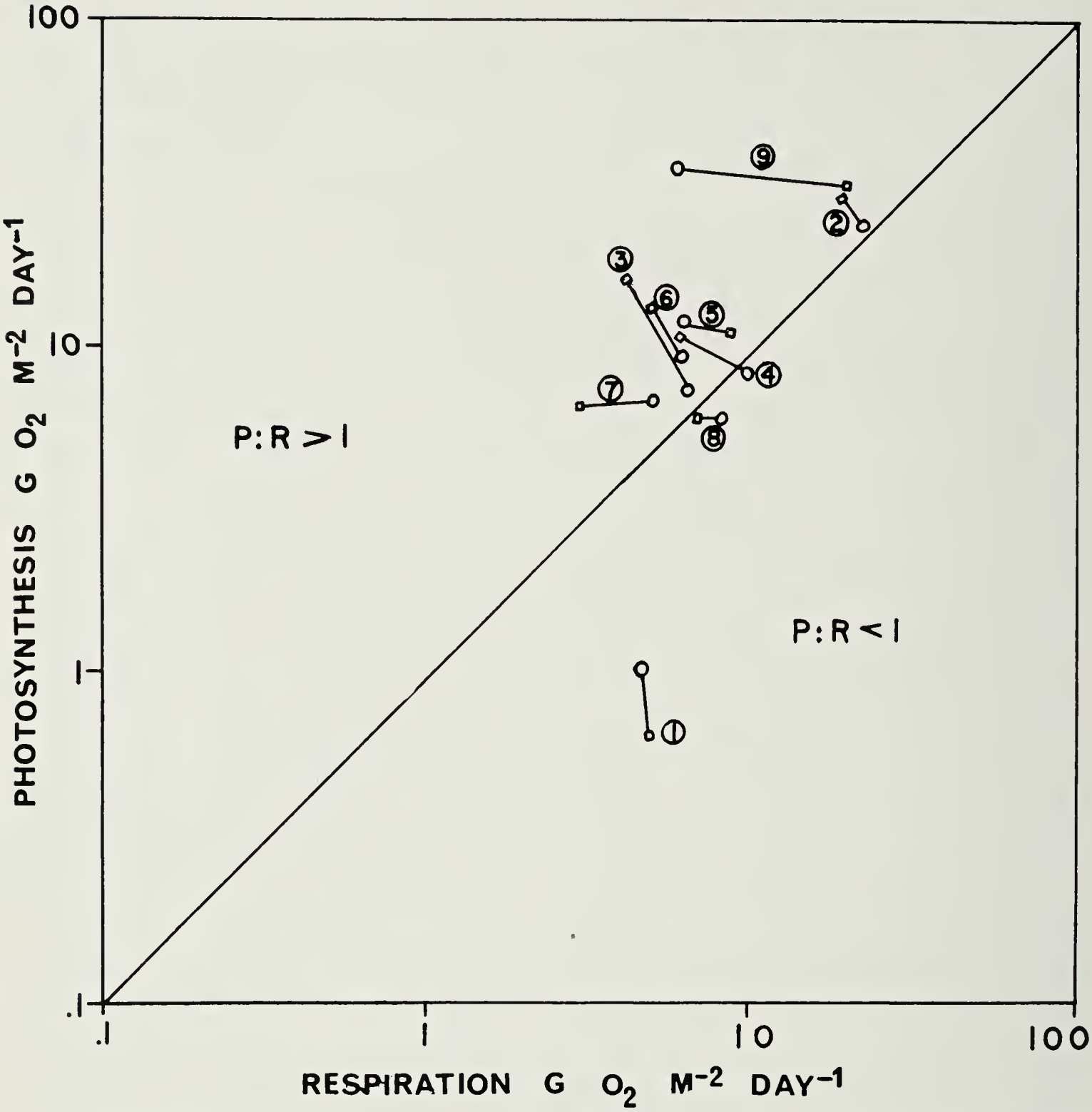

FIGURE 7. Range and relative dominance of autotrophic and heterotrophic metabolism in Worley Lake during summer. (1) = River in flood stage; (2) = Stable; (3) River approaching flood stage; (4) Stable; (5) Stable; (6) Stable; (7) Stable; (8) Stable but extremely turbid; (9) River in Third week of flood stage. (o) = South Station; (□) = North Station.

1959). During summer, the P/R ratio was usually greater than unity and productivity exceeded respiration at both stations except when the community was disturbed by winds or flood conditions (Fig. 7). Community metabolism, as indicated by the P/R ratio, may also serve as an indicator of the degree of community stability (Odum, 1959). In a study of autotrophic succession in the San Marcos River, San Marcos, Texas, Hannan (1967) reported a range from 0.59 to 1.50 in the P/R ratio. In the immature community, fluctuation in light intensity caused greater fluctuation in the P/R ratio than in the more mature community.

Acknowledgments

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NEW DISTRIBUTION RECORDS FOR FIVE MAMMALS OF THE COASTAL PLAIN OF NORTH CAROLINA

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ABSTRACT. — New locality records are presented for five species of rodents from eastern North Carolina.

Few published distribution records exist for mammals in the coastal plain of North Carolina. Brimley (1944 to 1946) and Smith, Funderburg and Quay (1960) summarized the statewide distributions for all species of mammals but did not elaborate on those from the coastal plain region. Recently Paul (1965) reported the first record of *Blarina telmalestes* from North Carolina (Hyde County), a lower coastal plain site. Paradiso (1960) described a new subspecies of *Peromyscus leucopus* from the Cape Henry, Virginia, area and commented on its probable distribution on the outer banks of North Carolina. Funderburg (1961) recorded the presence of the muskrat (*Ondatra zibethicus*) from New Hanover County. Aside from these scattered reports, little published knowledge exists relative to the mammalian fauna of this region.

It seemed worthwhile to present new information obtained from recent collections which modify currently understood distribution patterns. Hall and Kelson (1959) have summarized the distribution of North American mammals and it is relative to their distribution maps that these changes are suggested. These new records probably do not reflect changes in the established range but rather reveal the results of an increase in trapping effort and visual observations. The specimens referred to are deposited in the North Carolina State Museum (NCSM), Zoology Department Collections, North Carolina State University (NCS) or in various personal collections.

Marmota monax, woodchuck. Currently the nearest woodchucks are known only from the Coastal Plain of Virginia, immediately north of the North Carolina boundary. Sight records of woodchucks were reported to the author in 1961, 1962, and 1963 by W. L. Trent and Walter Gray, fisheries biologists working on the Roanoke River, Halifax and Hertford counties, North Carolina. Both individuals reported many sightings of this species in the spring of the year on both the north and south banks of the river and occasionally observed woodchucks swimming in the river. Two additional specimens are as follows: NCSM 256, Clayton, Johnston County; NCSM 257, Tillery, Halifax County. The Johnston County record (upper Coastal Plain) extends the known range of this species approximately 100 miles to the south.

Peromyscus leucopus, white-footed mouse. Rhoads and Young (1897) captured 12 specimens of *Peromyscus leucopus* from Chapanoke, Perquimanns County, and 10 specimens from Currituck, Currituck County. They noted that it was "the commonest species met with, being taken in all kinds of situations." Hall and Kelson (1959) list the Currituck records and Raleigh, Wake County, as peripheral. Although no other published records exist, the comment of Rhoads and Young (1897) on their abundance applies at least as far south as Hyde and Pitt counties, 50 to 75 miles south and east of the currently recorded range. This species is also common on the outer banks of North Carolina in Dare County (62 CLC, NCS uncatalogued). In this latter area and in the swampy regions of Hyde County, *Peromyscus leucopus* and *Peromyscus gossypinus* are commonly found together, while in drier sites only *Peromyscus leucopus* occurs. Additional records include: NCSM 127, 128, 129, and 343, Bertie County; NCSM 460, 461, 462, Greenville, Pitt County. Atkinson, Pender County, represents the southernmost record for *leucopus.* This specimen, NCS 1175, taken 4 December 1954, by James Burns.

Sigmodon hispidus, cotton rat. Hall and Kelson (1959) did not list the cotton rat from the coastal plain of North Carolina. Although its occurrence in Brunswick County, southeastern Virginia, Raleigh, North Carolina and the coastal regions of South Carolina was recorded. Brimley (1944-46) recorded the species from Beaufort, Brunswick and Craven counties, North Carolina and current records indicate the species to be common throughout the coastal plain area on the drier sites. Additional records include NCS 718, NCS 719, Camp Lejeune, Onslow County; NCSM 463, Greenville, Pitt County; NCSM 195 and 457, Wilmington, New Hanover County. The species is abundant in old fields throughout the upper coastal plain area.

Microtus pinetorum, pine vole. Hall and Kelson (1959) recorded no specimens of the pine vole from the coastal region of North Carolina with the exception of several voles taken in the northeasternmost county (Currituck. Hall and Kelson (1959) indicate a gap in the distribution of *M. pinetorum* in the Coastal Plain of North Carolina; the species occurring north, south and west of this region. The pine vole is generally uncommon in the coastal region, possibly because of the high water table and resulting wet habitat (Paul, manuscript in preparation) although it is locally numerous in some of the drier upper coastal plain sites. New records for this species include: NCSM 381, 3.8 mi. NNE South Mills, Camden County; NCS 306, Core Point, Currituck County; 1955-87 JBF (NCS uncatalogued), Carolina Beach, New Hanover County. This latter record was from a long leaf pine stand on a dry, sandy site. The maximum elevation in the Carolina Beach area is just a few feet above sea level. J. G. Boyette (pers. comm.) has taken large numbers of pine voles from the area around Falkland, Pitt County, an upland site.

Zapus hudsonius, meadow jumping mouse. The jumping mouse has

been collected in the Raleigh area by Brimley (1919) and more recently by Hester (Pers. comm.) near Wendell, Wake County, about 20 miles east of Raleigh. Hall and Kelson (1959), in following the monograph by Krutzsch (1959), have indicated that the probable range of *Zapus* includes most of the coastal plain region. Specimens were not examined by these authors except those taken from southeastern Virginia and the Raleigh area. Although it is possible that the jumping mouse does occur in this area, no record exists and its presence is somewhat doubtful. *Zapus* is apparently rare, even in the Raleigh area, and has seldom been taken in other regions of the Piedmont in North Carolina. Recent records (all Piedmont sites) include NCS 545, Raleigh, Wake County, and a captive specimen, not preserved, from Rougemont, Durham Co., was held by several students at North Carolina State University, July, 1946.

Acknowledgments

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ADDITIONS TO THE FLORA OF COLES COUNTY, ILLINOIS

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ABSTRACT. — A floristic study of a series of hillside marshes resulted in 56 plant species not previously recorded for Coles County, Illinois. Some of the species are extremely common but have been previously overlooked; others, however, are extremely rare, having been reported sporadically or not at all from East-central Illinois.

During the summers of 1967 and 1968 the authors undertook a study of a series of marshes located about five miles east of Charleston, Coles County, Illinois (NE¼, SW¼, Section 4, T12N, R10E). These marshes are located in a small valley which enters the Embarrass River just to the south of where Illinois Route 16 crosses the Embarrass River. Situated at an elevation of about 595 feet above sea level, these marshes will be flooded when the proposed Lincoln Reservoir is built.

All of the marshes are located in an area of about six acres and the marshes vary from about 200 to 4,000 square feet in size. Though not extensive, these marshes are composed of various ecological situations; and as a result, the flora of the area is relatively diverse. Some of this variation was discussed by Phipps and Speer (1958) who undertook a floristic study of the largest marsh in the area.

Most of the marshes are located on the northwest-facing hillside of the valley and slope to the west from a seep line about 10 feet above the valley floor. This seep line results from a gravelly deposit which overlays an impervious clay layer. At the southern edge of the marsh area are three small marshes (spot marshes), each about 200 square feet in size, that are fenced and have not been grazed for about 10 years. One hundred feet to the north is a more extensive, relatively flat marsh which is heavily grazed. Ten feet farther north is the largest of the marshes. This marsh is the one studied by Phipps and Speer (1958) and is extremely diverse. It consists of a steep hillside section, a low section in a depression at the base of the hill, and a high section which slopes about 10 degrees to the west from the seep line. No part of this marsh has been grazed since 1967, and the northern part was ungrazed when Phipps and Speer conducted their study. To the northeast of this large marsh are four small marshes, similar to the spot marshes to the south.

As a result of the present study, a number of plant species were found in and surrounding the marshes that have not been reported for Coles County, Illinois, by Jones and Fuller (1955) or Winterringer and Evers (1960). Of these, some are relatively common; others are extremely rare, having been reported sporadically or not at all from East-central Illinois. Included in this latter group are *Aster lucidulus, Carex*

sterilis, Eupatorium fistulosum, Pilea fontana, Rudbeckia sullivantii, Solidago patula and *Spiranthes gracilis.*

In the following list, the species followed by an asterisk were previously reported as occurring in Coles County by Phipps and Speer (1958) but were not incorporated into the checklist of Winterringer and Evers (1960). The monocots listed by Phipps and Speer were discussed by Ebinger (1967); and a reevaluation of the dicots in their list revealed that, of the new records they reported, three were identified incorrectly. Their *Epilobium adenocaulon* is *E. coloratum,* which is a new record for Coles County; their specimen labeled *Phlox glaberrima* is *P. divaricata,* while their *Helianthus annuus* should be referred to as the already reported *Rudbeckia hirta.* Phipps and Speer also listed *Viola cucullata* as a new record, but their specimen of this species could not be found.

The checklist which follows contains 56 vascular plants found at the hillside marsh that have not been recorded previously for Coles County, Illinois. The nomenclature of this list follows that of Jones (1963). Each species is followed by a brief note concerning the habitat in which it was collected, the collector's name and field number. All specimens are deposited in the herbarium of Eastern Illinois University.

CYPERACEAE

Carex festucacea Schk. In low marsh and moist lowland woods; Mueller 705.

Carex sterilis Willd. In high marsh and ungrazed pasture; Mueller 820.

Carex stipata Muhl. In all areas of the marsh; Parker 44.

Eleocharis erythropoda Steud. In grazed marsh; Mueller 819.

GRAMINEAE

Muhlenbergia mexicana (L.) Trin. In open pasture below marsh areas; Mueller 896.

LILIACEAE

Allium tricoccum Ait. Woods at edge of ungrazed spot marshes; Parker 75.

ORCHIDACEAE

Spiranthes gracilis (Bigel.) Beck Dry, grazed hillside above marsh area; Mueller 884.

BORAGINACEAE

Cynoglossum virginianum L. Wooded pasture below marshes; Mueller 832.

Lithospermum canescens (Michx.) Lehm. Dry eroded hillside above marsh; Ebinger 4296.

CAPRIFOLIACEAE

Lonicera prolifera (Kirchn.) Rehd. Vine on tree in shaded spot marsh; Parker 127.

Sambucus canadensis L. At edge of ungrazed spot marsh; Parker 123.

CARYOPHYLLACEAE

Cerastium vulgatum L. Open grazed pasture below marshes; Mueller 675.

Stellaria media (L.) Vill. Open, grazed pasture below marshes; Mueller 633.

CELASTRACEAE

Euonymus obovatus Nutt. Ungrazed, wooded hillside above marshes; Mueller 637.

COMPOSITAE

Aster lateriflorus (L.) Britt. In all marsh areas; Mueller 940.

Aster lucidulus (A. Gray) Wieg. In high, ungrazed marsh; Mueller 938.

Aster simplex Willd. Open, grazed pasture below marsh; Mueller 931.

Bidens frondosa L. Edge of low marsh; Mueller 904.

Cirsium vulgare (Savi) Tenore Open, grazed pasture below marshes; Mueller 794.

Eupatorium fistulosum Barratt Scattered throughout the ungrazed marsh areas; Mueller 898.

Helenium autumnale L. Edge of low marsh; Mueller 886.

Helianthus tomentosus Michx. Edge of stream below marshes; Mueller 944.

Krigia biflora (Walt.) Blake Dry, grazed hillside above marsh areas; Mueller 677.

Prenanthes alba L. Wooded, ungrazed hillside above marshes; Parker 115.

Prenanthes altissima L. Edge of stream below marshes in woods; Mueller 946.

Rudbecka sullivantii Boynt. & Beadle. Edge of low marsh and in moist pasture; Parker 111.

Solidago patula Muhl. Scattered throughout the ungrazed marsh areas; Mueller 902.

Solidago riddellii Frank. Edge of stream in pasture below marsh areas; Mueller 885.

CONVOLVULACEAE

Cuscuta gronovii Willd. Parasite on *Impatients* and *Solidago* in marsh areas; Parker 116.

CRUCIFERAE

Sisymbrium officinale (L.) Scop. Edge of stream in pasture below marsh areas; Mueller 679.

JUGLANDACEAE

Carya glabra (Mill.) Sweet. Woods above marsh; Mueller 848.

LABIATAE

Agastache nepetoides (L.) Ktze.* Drier part of spot marshes and pasture just below marsh; Ebinger 6494.

LEGUMINOSAE

Amphicarpa comosa (L.) G. Don.* Wooded, ungrazed hillside above marshes; Mueller 777.

Apios americana Medic.* Vine on *Salix discolor* in ungrazed high marsh; Mueller 775.

Desmodium cuspidatum (Muhl.) Loud. Open woods above marshes; Phipps and Speer 396.

Desmodium longifolium (T. & G.) Smyth. Lowland woods and pasture below marshes; Parker 118.

MORACEAE

Morus tatarica L. Open woods below marshes; Mueller 849.

ONAGRACEAE

Circaea latifolia Hill. Ungrazed woods below marshes; Parker 51.

Epilobium coloratum Muhl. In low marsh and hillside marsh; Mueller 782.

POLYGONACEAE

Polygonum punctatum Ell. In grazed marsh and low marsh; Mueller 905.

Rumex acetosella L. In grazed pasture below marshes; Mueller 811.

RANUNCULACEAE

Anemone virginiana L. Wooded, ungrazed hillside above marshes; Parker 37.

Clematis virginiana L. Edge of stream in open pasture; Mueller 774.

Thalictrum revoltum DC. Ungrazed open area at edge of stream below marshes; Mueller 665.

ROSACEAE

Agrimonia gryposepala Wallr. Ungrazed woods surrounding marshes; Mueller 743.

Fragaria virginiana Duch. In and just above hillside marsh; Parker 144.

RUTACEAE

Ptelea trifoliata L. Ungrazed woods below marsh; Mueller 662.

SCROPHULARIACEAE

Chelone glabra L.* Throughout the marsh areas; Mueller 906.

Dasistoma macrophylla (Nutt.) Raf. Edge of low marsh; Parker 42.

Lindernia anagallidea (Michx.) Pennell* In low marsh; Phipps and Speer 422.

Mimulus ringens L.* Scattered throughout most of the marsh areas; Mueller 763.

Veronica catenata Pennell* Standing water in marsh; Phipps and Speer 369.

SOLANACEAE

Physalis heterophylla Nees In open pasture below marsh; Ebinger 6495.

UMBELLIFERAE

Oxypolis rigidior (L.) Raf. Scattered throughout most marsh areas; Ebinger 6497.

Torilis japonica (Houtt.) DC. In moist pasture just below marshes; Parker 89.

URTICACEAE

Pilea fontana (Lunell) Rydb. Throughout all marsh areas; Mueller 876.

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VEGETATION SURVEY OF AN OAK-HICKORY MAPLE FOREST IN CLARK COUNTY, ILLINOIS

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ABSTRACT. — The present stand of the western-most part of Rocky Branch Nature Preserve, Clark County, Illinois, is composed of 136 stems per acre (above 4 inches d.b.h.) with a basal area of 99 square feet per acre. A total of 35 woody species are present on the site with white oak comprising half of the basal area and nearly one-third of the total individuals. Black oak, red oak, shagbark hickory, sugar maple, mockernut hickory, and bitternut hickory follow in order of Importance Value. Three broad cover types are recognized in the woodlot. The first area is a dry, relatively flat upland in which white oak has an Importance Value of over 100; the second is a mixed-hardwood region on the slopes and lowland areas where oaks, hickories, and sugar maple dominate, and the third is a sugar maple-dominated region on a steep north-facing hillside. The large number of saplings, seedlings, and small diameter trees of sugar maple indicates that its importance will increase in the woods.

The 130 acre Rocky Branch Nature Preserve is located about 6 miles northwest of Marshall in Clark County, Illinois. Because of the unique fauna and flora of this area, the Illinois Chapter of The Nature Conservancy purchased the tracks of land bordering parts of Rocky Branch Creek. This area is now under the trusteeship of Eastern Illinois University and is being maintained as a natural area for instructional and research purposes.

The woodlot represents the remnant of the typical forest associated with deep valleys of the Illinoian till of East-Central Illinois. The plants of the many sandstone walls, which border the creek, and the vegetation, which occurs in the ravines and on the slopes, make this an extremely botanically interesting area. In these areas are numerous species of plants that are not common to this part of Illinois. Found here are such woody plants as beech (*Fagus grandiflora*), tulip tree (*Liriodendron tulipifera*), red maple (*Acer rubrum*), and maple-leaved viburnum (*Viburnum acerifolium*). Herbaceous species that are near the extreme edge of their range include three species of sphagnum

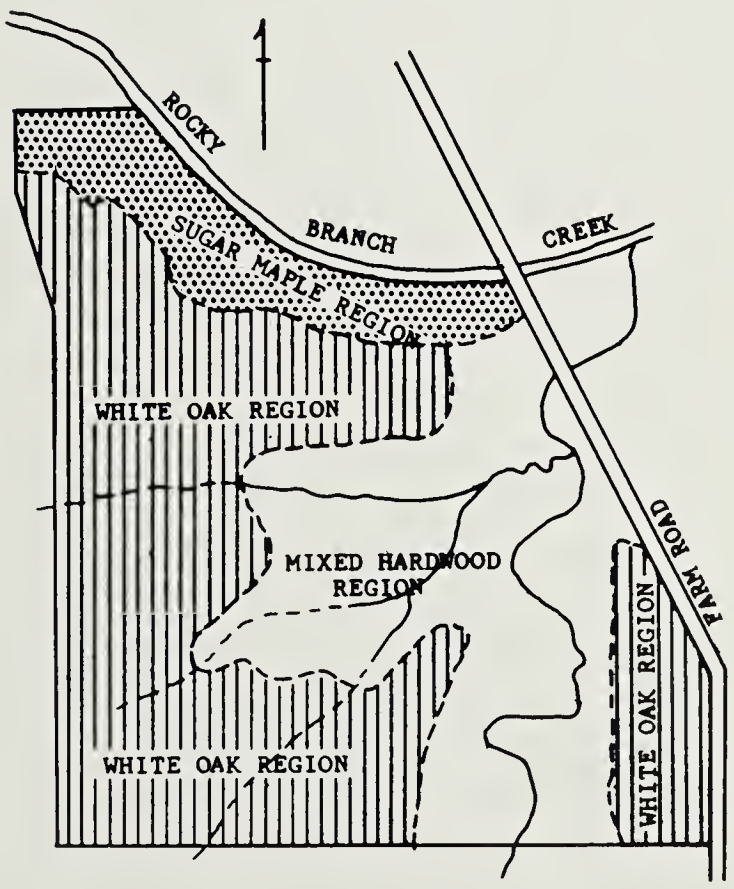

FIGURE 1. Map showing the distribution of the broad cover types in the western-most part of the Rocky Branch Nature Preserve.

moss (Arzeni, 1947), shining club moss (*Lycopodium lucidulum*), walking fern (*Camptosorus rhizophyllus*), partridge berry (*Mitchella repens*), beech drops (*Epifagus virginiana*), and bishop's cap (*Mitella diphylla*).

Prior to the acquisition of the land by The Nature Conservancy, the woods had been grazed to some extent and some timber had been cut. According to the local inhabitants, however, no extensive cutting has taken place since about 1900. A few stumps are still present in parts of the woods, indicating that more recent cutting has also occurred.

The only previous ecological work in the woodlot was conducted by Stover (1930), who completed a checklist of the plants and gave short descriptions of the plant associations that existed. The first complete inventory of the woody vegetation of the western part of this area was conducted in 1967, and the results are presented in this paper. The western-most section was studied since it represents the most mature and least disturbed part of the woods.

Description of the Woodlot

The area studied in this survey was the western-most part of Rocky Branch Nature Preserve, consisting of a 16 acre woodlot located in the NE¼, SE¼ of Section 30, T12N, R12W, Clark County, Illinois. This woodlot is on Illinoian till, 5 miles to the south of the terminal moraine of the Wisconsin glacier. The topography of the area is quite diverse. The western part is a level to gently sloping upland while the remainder of the woodlot is divided by ravines with gradually sloping sides to steep banks. The northern perimeter of the woods consists of outcroppings of sandstone and an accompanying severe bank overlooking Rocky Branch Creek. The west and south edges of the woodlot are limited by open fields, the east edge by a farm road, and have the usual edge vegetation of low shrubs and weed trees typical of fence rows. The overall relief is about 60 feet, the high point in the southwest corner being 645 feet above sea level.

Method of Study

The woodlot was divided into 50 meter square quadrats and the number, size, and species of trees above 4 inches d.b.h. were recorded in each. The diameters were recorded to the nearest 1/10 of an inch. The Importance Value (IV) was then calculated from the actual data to provide a better basis of comparison of the various species. As used here, the determination of the IV follows the procedure outlined by McIntosh (1957) and later by Boggess (1964), in which the IV is the sum of the relative density

$$\frac{\text{(number of individuals of a species}}{\text{(number of individuals of all species}} \times 100)$$

and of the relative dominance

$$\frac{\text{(basal area of a species}}{\text{(total basal area of all species}} \times 100).$$

Also, the IV was calculated for each quadrat to determine if different vegetation zones existed in the woods. Dead-standing and dead-downed trees also were measured and identified when possible. A record

was kept of the amount of coppice growth that existed in the woodlot, as well as the number and size of cut stumps.

In each 50 meter quadrat, four 1/100 and 1/1000 acre nested, circular plots were randomly located. The saplings (1 to 4 inches d.b.h.) were recorded on the larger plot and the seedlings on the smaller. The seedlings were divided into those under one foot in height and those over one foot in height but less than one inch in diameter.

The taxonomic nomenclature used in this paper follows that of Jones (1963).

Results and Discussion

A total of 35 woody species were recorded on the woodlot. Of this total, 23 were canopy trees, 6 were understory trees, and 6 were woody shrubs and vines. The species encountered, with their density and frequency by height and diameter class, appear in Table 1. The 14 leading species encountered, with their relative values, average diameters, and number of individuals per acre in broad diameter classes are included in Table 2.

Three broad cover types are recognized in the area, based on the Importance Values of the leading species on each of the quadrats studied. Quadrats of similar composition were grouped together and the cover types were defined, based on the Importance Value level of white oak. The cover-type boundaries, shown in Figure 1, were smoothed by field observation.

White oak. The dry, relatively flat uplands on the west and southeastern edges of the woods are dominated by white oak which accounts for over 50 per cent of the total Importance Value of the areas (IV of 100 or more). Black oak and shagbark hickory are the cost common associates and have higher Importance Values than any other secondary species. Red oak, mockernut hickory, bitternut hickory, black walnut, slippery elm, and white ash have high frequencies, while sugar maple and pignut hickory are present only about 50 per cent of the time. All eight of these species have very low Importance Values. In the southern part of the western upland area, no sugar maple occurs, but near the steep, northern slope its Importance Value is 15. Also, the frequency and density of saplings and seedlings of sugar maple and ironwood increase greatly in the northern part of this area.

Sugar maple. The steep, north-facing hillside which overlooks Rocky Branch Creek is dominated by sugar maple, red oak, and to a lesser extent, bitternut hickory, basswood, beech, and ironwood. Sugar maple comprised 20 to 40 per cent of the stand and its IV averages 70. Red oak is the second most important species with an IV of 35. Nearly all of the basswood, beech, and ironwood are restricted to this part of the woods, and bitternut hickory has a much higher Importance Value here than in the remainder of the woodlot. A few individuals of black walnut, mockernut hickory, slippery elm, and white ash occur here, but have very low Importance Values. Very little white and black oak occur in this cover type.

Mixed hardwood. The remainder

of the woods consists of gentle to relatively-steep slopes and lowland areas associated with the small stream and the few ravines that occur in the woodlot. White oak dominates this cover type with an average IV of about 65 and is first in Importance Value in all quadrats. Black oak, red oak, and shagbark hickory are the most common associates with Importance Values between 15 and 24. Black walnut, mockernut hickory, and bitternut hickory have high frequencies but low Importance Values, while sugar maple is more important here than in the upland areas. On some of the steeper northern and northwestern slopes of this area, a few beech occur and the amount of sugar maple increases. Associated with the small terraces near the stream are a few individuals of butternut, persimmon, slippery elm, red mulberry, and sassafras.

Of the aborescent species found at Rocky Branch, the oaks (white, black, and red) rank first, second, and third in importance, respectively, and comprise the most important species group in the woodlot. White oak, which has an IV of 80 in the woods, comprises half of the basal area and nearly one-third of the total individuals in the area studied. It exceeds the other species in all broad diameter classes except the 4- to 6-inch class. This species is the leading dominant in all of the sample quadrats except on the steep, north-facing slope adjacent to Rocky Branch Creek.

Both black and red oak have an IV of about 20 in the woodlot. Black oak usually ranks second to white oak in the uplands of the woodlot, while red oak is more common on the slopes. Both species have poor size-class distributions, with the majority of the individuals occurring in the 7- to 12 and 13- to 24-inch classes. Neither species is well represented in the sapling and seedling classes and are more poorly represented than white oak. The average diameter of 15.2 for black oak and 13.1 for red oak qualify them as the largest species in the woodlot. The largest individual in the woods is a 34-inch black oak.

Shagbark, mockernut, bitternut, and pignut hickories rank third, fifth, sixth, and eighth in importance, respectively, and comprise the second most important species group in the natural area. Shagbark, mockernut, and bitternut hickories are well distributed throughout the woodlot with frequencies of 100 per cent, while pignut is much more restricted, occurring in only 56 per cent of the quadrats examined. All of the hickories have good size-class distribution with most of their individuals in the 4- to 6-inch class and decreasing upward. Bitternut hickory is the only species of this genus with individuals in the 25- to 36-inch class. Shagbark hickory exceeds all other species in the 4- to 6-inch diameter class with 65 per cent of the individuals in this class. This species ranks second to white oak in total individuals per acre.

Sugar maple ranks fifth in importance and is commonly associated with third-ranked red oak. The high importance of sugar maple is due to tree number rather than tree size, as 65 per cent of this species occur in the 4- to 6-inch diameter class. Although not found throughout the

woods, its high importance on the north-facing hillside adjacent to Rocky Branch Creek and its occurrence in the lowland and on the other slopes of the woods indicate the more mesic nature of this species. Sugar maple ranks first in the number of seedlings and saplings per acre, having more than twice the density as the next most important species. Ironwood, which occurs as an understory tree that is associated with sugar maple, ranks thirteenth in importance. This species has nearly the same distribution in the woodlot as sugar maple and ranks third in seedlings and fourth in saplings per acre. Similar association of ironwood and sugar maple was observed by Ebinger (1968) in Sargents Woods, located on the Shelbyville Moraine.

Black walnut, slippery elm, white ash, and sassafras are scattered throughout the stand, but have Importance Values less than 5. No saplings or seedlings of black walnut occurred in the sample plots and its poor size-class distribution indicates that this species will decrease in importance in the future. The remaining three species, slippery elm, white ash, and sassafras, have a much better size-class distribution with over half of their individuals in the 4- to 6-inch diameter class. White ash ranks second in seedlings per acre, but the small number of saplings and trees of this species indicates its poor survival value. Slippery elm, in contrast, ranks second in saplings per acre. Its importance in the woods will probably not increase, however, due to mortality from phloem necrosis and Dutch elm disease.

Beech (fourteenth in IV) has a very restricted distribution in the woods, being found only on relatively steep slopes with a northern or northwestern exposure. Seedlings and saplings of beech are also restricted to these areas. This mesic site preference and the small number of seedlings and saplings indicate that the importance of this species will probably not increase in the woodlot.

A total of 31 stumps, with a total basal area of 41 square feet, was found in the woods. Most of these had been cut from the upland areas on the west and east edges of the woodlot and it appears that most of the trees cut were species of oak. Coppice growth is not an important feature of the woodlot, but it may give an indication as to when the last major cutting took place. Less than 4 per cent of the trees were composed of two or more main stems and accounted for less than 5 per cent of the basal area of the stand. Increment borings of the coppice stems showed an average stem age of 62 years.

The number of dead-standing and dead-downed trees is not an important feature of the woodlot. The total mortality of all species is only 8.6 trees per acre with a basal area of 3.0 square feet per acre. Dead white oaks average 3.6 trees per acre with a basal area of 1.3 square feet per acre. Dead elms averaged 1.1 trees per acre with a basal area of 0.4 square feet per acre. This is extremely low, particularly when compared with woods on the Shelbyville Moraine and farther north. In Sargents Woods (Ebinger, 1968) and Baber Woods (McClain and Ebinger,

1968) located on the Shelbyville Moraine, dead elms averaged 3 to 5 trees per acre with a basal area of 1 to 2 square feet per acre. All other species had a mortality of less than 0.7 trees per acre and less than 0.5 square feet of basal area per acre.

White oak and the hickory species are reproducing fairly well in the woodlot and have good size-class distribution. This indicates that these species represent a relatively stable community and that their importance in the stand will continue. In contrast, black and red oak have poor size-class distribution and will probably decrease in importance as mortality occurs in the larger diameter classes.

The more mesic hardwoods, such as sugar maple, white ash, basswood, and beech, are more restricted in the woodlot and except for sugar maple are not making substantial advances in the dried upland. The amount of sugar maple found and its distribution are important features of the area. The large number of seedlings and saplings, the good size-class distribution, and the ability of sugar maple to take advantage of canopy openings that occur, indicate its increasing importance in the woods. The number of sugar maple seedlings (4453 per acre) is almost as great as for all other tree species combined. This trend toward a more mesic woodland situation was observed by McClain and Ebinger (1968) and by Ebinger (1968) in other upland forests in East-Central Illinois.

Acknowledgments

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TABLE 1.—Density Per Acre and Frequency of Woody Species by Height or Diameter Class. The species symbol will be used to identify species in subsequent tables and figures.

Scientific Name	Common Name	Symbol	Height Class					Diameter Class			
			Density			Frequency %		1″-4″		4″ +	
			<1′	>1′<1″ dbh	Total	<1′	>1′<1″ dbh	Density	Frequency %	Density	Frequency %
Quercus alba L.	White oak	WO	109	31	140	9.4	3.1	5	4.7	45.75	100
Quercus velutina Lam.	Black oak	BO	46	62	108	4.7	6.3	.....	.....	9.56	100
Quercus rubra L.	Red oak	RO	15	62	77	1.6	3.1	2	1.6	10.44	88
Carya ovata (Mill.) K. Koch	Shagbark hickory	SH	15	47	62	1.6	4.7	16	14.1	13.75	100
Acer saccharum Marsh.	Sugar maple	SM	3,781	672	4,453	62.4	32.8	131	56.3	10.81	88
Carya tomentosa (Poir.) Nutt.	Mockernut hickory	MH	15	94	109	1.6	7.8	11	9.4	7.63	100
Carya cordiformis (Wang.) K. Koch	Bitternut hickory	BH	171	62	233	12.5	6.3	14	14.1	6.94	100
Juglans nigra L.	Black walnut	BW	.....	.....	.....	.....	.....	.....	.....	4.44	88
Carya glabra (Mill.) Sweet	Pignut hickory	PH	125	62	187	12.5	6.3	14	12.5	5.31	56
Ulmus rubra Muhl.	Slippery elm	SE	266	422	688	23.4	26.6	63	32.8	4.56	81
Fraxinus americana L.	White ash	WA	1,922	125	2,047	48.4	10.9	16	12.5	3.06	50
Sassafras albidum (Nutt.) Nees	Sassafras	Sa	47	109	156	4.7	7.8	.....	.....	3.06	75
Ostrya virginiana (Mill.) K. Koch	Ironwood	Ir	859	343	1,202	40.6	26.6	34	23.4	2.81	63
Fagus grandifolia Ehrh.	Beech	Be	.....	31	31	.....	3.1	3	3.1	1.13	50
Quercus imbricaria Michx.	Shingle oak	SO	.....	.....	.....	.....	.....	.....	.....	1.44	25
Prunus serotina Ehrh.	Black cherry	BC	234	171	405	15.6	15.6	2	1.6	1.63	31
Tilia americana L.	Basswood	Ba	.....	.....	.....	.....	.....	.....	.....	0.75	19
Quercus palustris Muench.	Pin oak	PO	.....	.....	.....	.....	.....	.....	.....	0.50	6
Carpinus caroliniana Walt.	Muscle tree	MT	422	125	547	18.7	9.4	22	15.6	0.69	38
Cornus florida L.	Flowering dogwood	FD	234	203	437	9.4	12.5	72	34.4	0.63	38

TABLE 1.—Density Per Acre and Frequency of Woody Species by Height or Diameter Class. The species symbol will be used to identify species in subsequent tables and figures. (Concluded)

Scientific Name	Common Name	Symbol	Height Class					Diameter Class			
			Density			Frequency %		1″-4″		4″ +	
			<1′	>1′<1″ dbh	Total	<1′	>1′<1″ dbh	Density	Frequency %	Density	Frequency %
Morus rubra L.	Red mulberry	RM	.....	.....	.....	.....	.....	8	7.8	0.56	25
Nyssa sylvatica Marsh.	Black gum	BG	15	15	30	1.6	1.6	2	1.6	0.31	12
Celtis occidentalis L.	Hackberry	Ha	.....	.....	.....	.....	.....	.....	.....	0.25	25
Ulmus americana L.	American elm	AE	.....	.....	.....	.....	.....	.....	.....	0.13	12
Juglans cinerea L.	Butternut	Bu	.....	.....	.....	.....	.....	.....	.....	0.13	6
Cercis canadensis L.	Redbud	Re	31	.....	31	1.6	.....	.....	.....	0.13	6
Diospyros virginiana L.	Persimmon	Pe	.....	15	15	.....	1.6	.....	.....	0.06	6
Platanus occidentalis L.	Sycamore	Sy	.....	.....	.....	.....	.....	.....	.....	0.06	6
Rhus radicans L.	Poison ivy	PI	1,500	828	2,328	18.7	17.2	.....	.....	.....	.....
Corylus americana Walt.	Hazelnut	Hz	78	158	236	4.7	6.3	.....	.....	.....	.....
Celastrus scandens L.	Bittersweet	Bi	453	187	640	18.7	10.9	.....	.....	.....	.....
Hydrangea arborescens L.	Wild hydrangea	WH	31	47	78	1.6	3.1	.....	.....	.....	.....
Amelanchier arborea (Michx. f.) Fern	Juneberry	Ju	47	.....	47	3.1	.....	.....	.....	.....	.....
Sambucus canadensis L.	Common elder	CE	31	15	46	1.6	1.6	.....	.....	.....	.....
Crataegus margaretta Ashe.	Hawthorn	Hw	.....	62	62	.....	4.7	.....	.....	.....	.....
Total	.....	.....	10,447	3,948	14,395	.....	.....	415	.....	136.52	.....

TABLE 2. — Number of Trees, Basal Area per Acre, Importance Value Index and Average Diameter for Leading Dominants.

Species	4-6		7-12		13-24		25-36		Total		Percent Total		I.V.I.	Av. Diam., In.
	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	Rel. Den.	Rel. Dom.		
WO	7.00	1.19	17.69	10.15	20.19	32.18	0.88	3.50	45.76	47.02	33.52	47.40	80.92	12.7
BO	1.25	0.23	3.06	1.77	4.63	8.54	0.63	2.79	9.57	13.33	7.01	13.44	20.45	15.2
RO	2.56	0.39	2.69	1.38	4.81	8.46	0.38	1.68	10.44	11.91	7.65	12.01	19.66	13.1
SH	9.00	1.34	4.19	1.87	0.56	0.65	.....	.....	13.75	3.86	10.08	3.90	13.98	6.7
SM	6.06	0.88	3.18	1.52	1.44	2.13	0.13	0.43	10.81	4.96	7.92	5.01	12.93	8.0
MH	3.88	0.64	2.88	1.41	0.88	1.07	.....	.....	7.64	3.12	5.59	3.14	8.73	7.9
BH	3.25	0.51	3.00	1.47	0.63	1.02	0.06	0.34	6.94	3.34	5.08	3.38	8.46	7.9
BW	0.81	0.15	2.75	1.69	0.88	1.06	.....	.....	4.44	2.90	3.25	2.93	6.18	10.5
PH	2.36	0.37	2.50	1.19	0.44	0.55	.....	.....	5.30	2.11	3.89	2.14	6.03	8.0
SE	3.19	0.42	1.19	0.52	0.19	0.19	.....	.....	4.57	1.13	3.34	1.14	4.48	6.3
WA	1.88	0.27	1.06	0.49	0.13	0.15	.....	.....	3.07	0.91	2.24	0.92	3.16	6.9
Sa	2.25	0.31	0.75	0.28	0.06	0.15	.....	.....	3.06	0.74	2.24	0.75	2.99	6.1
IW	2.31	0.33	0.50	0.16	.....	.....	.....	.....	2.81	0.49	2.06	0.49	2.55	5.5
Be	0.50	0.06	0.31	0.20	0.25	0.45	0.06	0.35	1.12	1.06	0.82	1.07	1.89	10.7
Others	4.75	0.64	1.94	0.95	0.56	0.68	.....	.....	7.25	2.27	5.31	2.28	7.59	.....
Total	51.05	7.73	47.69	25.05	35.65	57.28	2.14	9.09	136.53	99.15	100.00	100.00	200.00	.....

EFFECTS OF INGESTED GOSSYPOL ON THE DUODENUM OF RATS

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ABSTRACT. — A significant increase in the mitotic index and rate of cell extrusion occurred in the duodenum of rats fed gossypol-containing cottonseed meal for 30 days. No histopathological effects in the mucosa, submucosa or muscularis were observed.

Limitations have been imposed upon the use of cottonseed meal as a food source because of the presence of a toxic phenolic pigment, gossypol. This substance is highly toxic to non-ruminant animals and has been shown to accumulate in the organs of some animals (Sharma et al, 1966; Smith and Clawson, 1965). The variability in the toxicity of gossypol suggests that there is likely a greater intestinal absorption of gossypol rather than an effect on variation in the metabolic reactions of the animals (Bressani et al, 1964). The manner in which gossypol is absorbed by the intestinal mucosa and transported across the intestinal wall is presently under study in our laboratory. The effect of ingested gossypol on the cell dynamics of the duodenal region of the small intestine of rats was undertaken to determine any histological changes that may influence absorption studies.

MATERIALS AND METHODS

The animals used in this study were from a colony of CF-E rats maintained in this laboratory. Twenty-four male rats (30 days of age) were randomly selected, housed individually, and allowed food and water *ad libitum*. Twelve control rats were fed a commercial rat diet in meal form and twelve experimental rats were fed the commercial diet containing 20% cottonseed meal by weight. The cottonseed meal contained 0.26% free gossypol and 0.92% total gossypol. All animals were maintained on diets for 30 days.

Rats were killed between 8:00 and 9:00 a.m. by cervical dislocation. The abdomen was opened immediately, and a section of the duodenum approximately one-half to three-fourth centimenter long was removed about five millimeters posterior to the pylorus. Pieces of the duodenum were fixed overnight in 10% neutral formalin. After fixation, the tissues were dehydrated in ethanol, cleared in xylene, and embedded in a mixture of 90% paraffin, 10% bayberry wax at 58C. Cross sections of the duodenum were cut at six microns and stained with Harris' hematoxylin and eosin.

All stages of mitosis of the epithelial cells lining the crypts were counted. Only those crypts which were sectioned throughout their entire length were used for mitotic counts. The number of interphasic

and mitotic epithelial cells in histological sections was determined by counting their nuclei. The nuclei of chief and goblet cells were included in the counts, but not those of migrating lymphocytes and Paneth cells. All counts were made with oil immersion (970X). The number of mitotic figures, interphasic nuclei, and total nuclei were recorded for 40 crypts for each animal.

In determining the percentage of villi with extrusion zones, only those villi were examined in which the section passed through the tip, as shown by the presence of a continuous single layer of nuclei in the epithelium. Forty to sixty villus tips were examined per animal and classified as to whether they did or did not have extrusion zones. The results are expressed as the per cent of villi having extrusion zones.

Results and Discussion

The mitotic indices of the crypt cell population of the rat duodenum are presented in Table 1. There is a significant ($P < 0.01$) increase in the mitotic index of the duodenual crypt cell nuclei in the experimental animals. Mitosis normally occurs only in the cells lining the crypts. In the present study, mitotic figures were observed only in spaces at the base of the villi.

The percentage of villi having extrusion zones in the experimental animals is significantly ($P < 0.01$) greater than in the controls (Table 2).

It has been shown that the epithelial cells on the villus originate from proliferating cells in the crypt of Lieberkühn and then migrate up the sides of the villus to be extruded at the apex of the villus (Leblond and Messier, 1958). A delicate balance is believed to exist between the rate of cell proliferation in the crypts and the number of cells being extruded at the apex of the villus. In experiments conducted on rats, Leblond and Steven (1948) determined that 1.57 days were required for a cell to migrate from the crypt to the apex of the villus in the duodenum.

The increased mitotic index and presence of villi with extrusion zones in the duodenum of gossypol-fed rats indicate a rapid turnover of intestinal epithelium. This greater turnover of intestinal epithelium among the experimental animals suggests several possible effects of ingested gossypol. The rapid turnover may serve to renew the mucosal lining from trauma of gossypol and gossypol derivatives found in the cotton-

Table 1. — Mitotic Index (M.I.) of the Duodenum in Male CF-E Rats.

Diet	Number of Rats	M.I. (%)[1]
Control	12	5.41±.26
Experimental	12	7.53±.17[2]

[1] Mean ± standard error
[2] $P < 0.01$

Table 2. — Mean per cent duodenal villi with extrusion zones in male CF-3 rats.

Diet	Number of Rats	Villi With Extrusion Zones (%)[1]
Control	12	34.2±.32
Experimental	12	40.3±.27[2]

[1] Mean ± standard error
[2] $P < 0.01$

seed meal. Further, this increase may provide extra enzymes necessary for the hydrolysis of cottonseed meal.

Rats fed cottonseed meal did not exhibit any histopathological effects such as inflamatory reactions and necrotic erosion of the mucosa. The submucosa and muscularis appeared histologically normal among the experimental animals.

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A FACTORIAL ECOLOGY OF ROCKFORD, 1960

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ABSTRACT. — Rockford's industrial geography has been studied by Alexander, Harris, Forstall, and other geographers. Less attention has been given to the social geography of this second largest city in Illinois. The purpose of this paper is to provide a scientifically-based picture of some dimensions of Rockford's social space. Proximity to Chicago is of prime importance in this study, for the Chicago model is utilized as a yardstick in this comparative analysis. Rockford's ecological patterns are shown to mock those of Chicago.

That Rockford's history is not unlike the history of Chicago is easily demonstrated. Rockford started as a transport center and became a manufacturing city. It differs from Chicago in the obvious fact that it never grew to several-million proportions and hence never attained the financial importance of Chicago.

The broader aspects of Rockford's development center about the city's position upon the Rock River midway between Galena and Chicago. In fact, Rockford started as a ford on the Rock River, and it was originally called "Midway" (Church, 1900). The Galena lead mines were opened in 1824, and ten years after that settlement started in what is now Rockford. It was four days travel time by coach or wagon from Galena to Fort Dearborn, and the opportunity developed for enterprising individuals to start a roadhouse and a ferry at the ford.

The town grew on both sides of the Rock River, and the shallow water areas eventually supported dams. Industries sought sites on both banks of the Rock River and the Galena and Chicago Union (soon to become part of the Chicago and North Western) Railroad came to Rockford in 1852. This cut down travel time to Chicago considerably. Telephones reached Rockford by 1879, and street railway service was in operation by 1881 (State of Illinois, 1941). The advent of street railways greatly increased internal mobility of workers as industry flourished.

After the cost of bridges became more bearable, it was increasingly apparent (even in those early days) that the west bank of the river would be the more industrially successful side of town. Irish and Swedish immigrants had been there several decades, and as they became more affluent they began to spread over the town from their western and southern neighborhoods. Meanwhile the descendants of the town founders had located themselves on the west bank of the river to the north of the commercial core (Pierce, 1887).

By the turn of the century Rockford continued to attract new industries and the established ones grew. It was also possible to move away from the river and still have energy to run machines. By 1956 industries were all over town—except for the high status residential

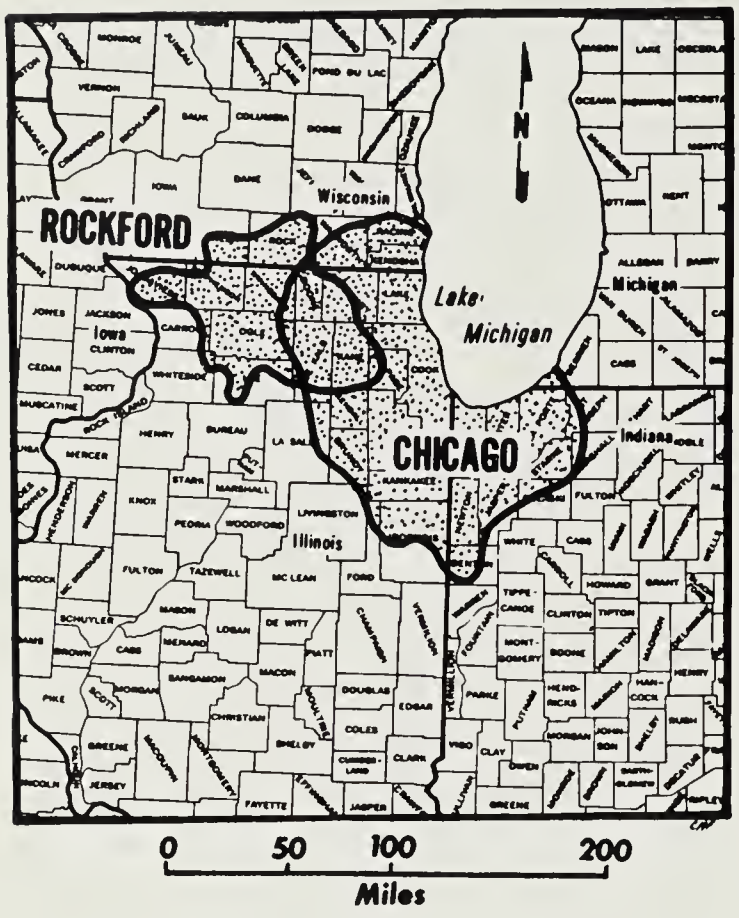

Figure 1. The Chicago and Rockford Labor Markets

areas (Kincaid and Associates, 1957). Most of the settled industrial sites are however, within walking distance of older blue collar neighborhoods.

By 1960, people were commuting to Rockford for work from a labor market which encompassed a vast portion of northern Illinois (Berry, 1967). In addition, Rockford's commuting field overlapped significantly with that of Chicago, indicating strong metropolitan ties. Data from the 1960 Census of Population and Housing help demonstrate these ties.

The Factorial Ecology

Methodology. An extensive review of factorial ecology and its evolution from social area analysis was recently completed by Rees (1968). In the earlier contributions of Shevky and Bell (1955) three social indices comprising constructs derived from census tract data were formulated. These indices are socio-economic status, stage in life cycle, and minority (usually Negro) segregation. Subsequent studies, Rees concludes, confirm the validity of these constructs, and also reveal that each index is characterized by a distinct social-spatial pattern. Areas of high, medium, or low socio-economic status tend to radiate outward in sectors from the commercial core. Zones of similar age structure, family composition, and housing type tend to form outward from the city center in concentric rings. Minority groups concentrate in segregated neighborhoods. Rees goes on to agree that these findings effectively integrate the growth theories of Burgess and Hoyt and the studies of segregation of the Duncans and Taeubers. The social area analyses of Van Arsdol, Camilleri, and Schmid (1958) are perhaps most relevant to this study because American cities similar to Rockford are examined.

Modern computer technology has recently allowed social area analysis to evolve into factorial ecology. With factor analysis programs it is possible to reduce an extensive n-census tract x m-variable matrix to relevant dimensions quickly and cheaply.

This is the essence of the Rees study, for in addition to the three social area analysis indices, other factors that we present are isolated quickly, and prepared for further study. Furthermore, it is possible to demonstrate the cross-cultural utility of factorial ecology. Berry and Rees (1969) identify patterns of familism, literacy, ethnicity, social status, and several other dimensions in a study

TABLE 1. — Rotated Factors, Varimax Convergence.

Variable[a]	Communality (h^2)	FACTOR MATRIX I	II	III
Percent over $10,000	0.903	0.942	0.015	0.124
Percent substandard	0.929	-0.940	-0.187	-0.106
Percent white collar	0.893	0.909	-0.186	-0.176
Median income	0.894	0.885	0.232	-0.240
Percent unemployed	0.846	-0.883	-0.071	0.243
Median school years	0.793	0.851	0.079	-0.250
Percent under $3,000	0.894	-0.700	-0.551	0.318
Percent under 18	0.944	0.036	0.964	0.118
Population per household	0.959	0.079	0.957	0.194
Percent over 65	0.947	0.053	-0.930	0.282
Percent structures after 1950	0.736	0.281	0.641	-0.497
Percent Negro	0.758	-0.559	0.089	0.662
Cumulative per cent of common variance		55.0	89.0	100.0

a. Based on 1960 census tract data, U. S. Bureau of Census, Census of Population and Housing, 1960.

of Calcutta of 1961 through factor analysis.

The Rockford Data. For purposes of comparison with Rees' earlier study of Chicago, twelve variables with the most central significance in that study were assembled for the census tracts of metropolitan Rockford, and analysed using a factor analysis program available on The University of Chicago 7040-7094 IBM computer system. The variables are shown on Table 1.

Initial analysis of the 12 variable by 43 census tract matrix for the SMSA of 1960 encountered certain difficulties due to the economic nature of surrounding rural tracts. A reduced 12 x 37 matrix produced more refined details of the urbanized Rockford tracts for analysis.

The several steps of factor analysis show how the variables relate to one another: For example, the 12 x 12 matrix of correlation coefficients that is prepared before the factoring is undertaken reveals that percent white collar workers correlates highly with percent of families with annual incomes exceeding $10,000 (0.845) and with educational level (0.885). Conversely, the percent with incomes over $10,000 shows high negative correlations with percent of housing substandard (-0.841) and percent unemployed (-0.833). Percent of population under 18 years correlates very highly with population per household (0.978) and the former variable shows a high negative correlation with population over 65 years (-0.845). These statistical implications of the strength of the socio-economic status (SES) and life cycle (LC) dimensions are demonstrated clearly by the factoring procedures.

This next (factoring) step in the computer program produces an m x r matrix by the principal components method. In the Rockford example, two principal components accounted for 81.7 percent of the variance present in the original twelve variables, showing that a 37 x 2 matrix tells almost as much about Rockford's so-

cial geography as the original 37 x 12 matrix. This two factor principal axis solution is shown on Figure 2. The negative and positive SES and LC variables are evident.

A Negro segration (NS) factor was separated from the other factors in a subsequent factoring operation so that a three factor solution resulted (37 x 3 matrix). Normal

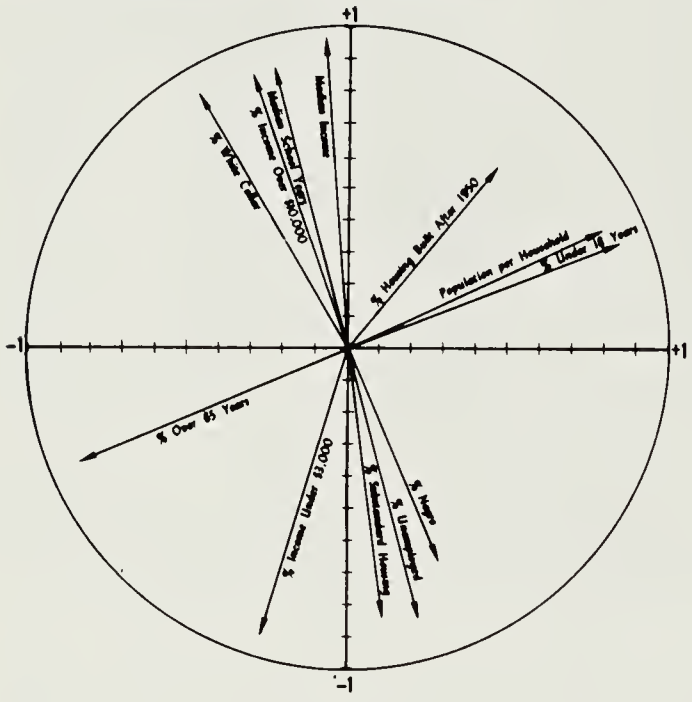

Figure 2. The Principal Components

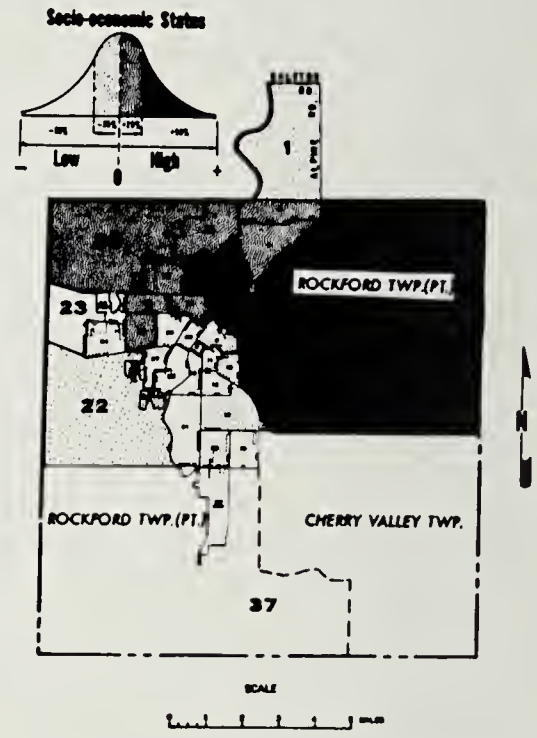

Figure 3. Factor I-Socio -economic Status

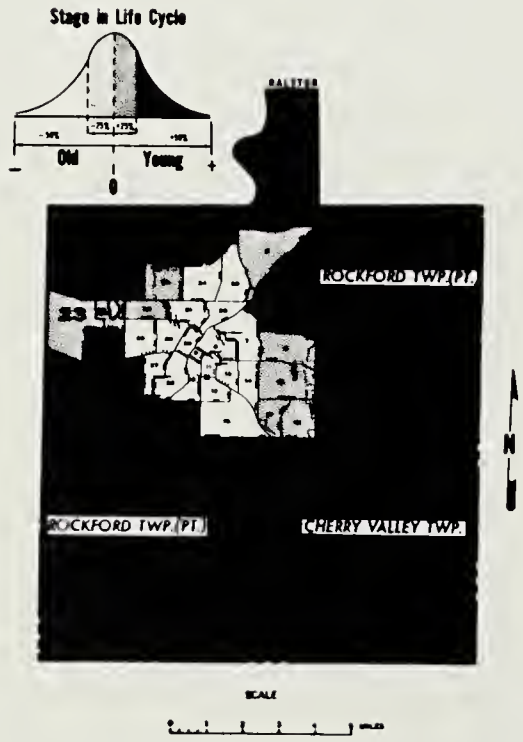

Figure 4. Factor II-Stage in Life Cycle

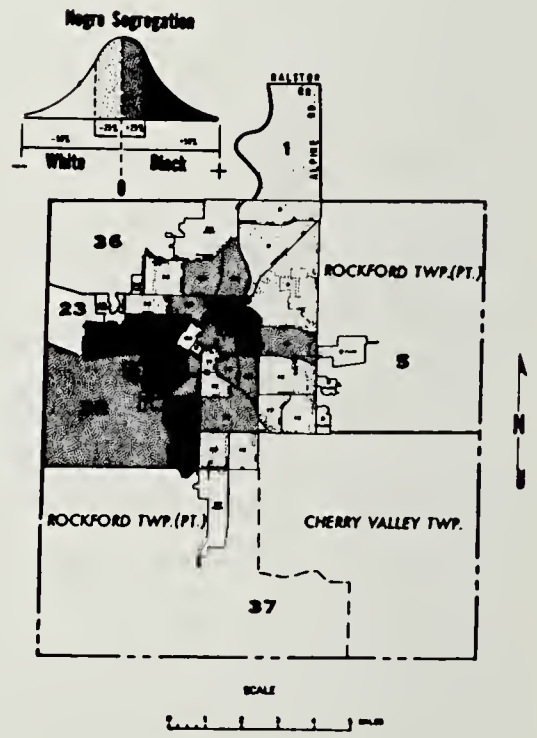

Figure 5. Factor III-Negro Segregation

PLATE I.

varimax rotation of all factors with eigenvalues exceeding 0.6 was used to produce the factor loadings shown on Table 1. Although the NS variable comprising Factor III was not strong enough to show independence when eigenvalues greater than unity were used, all three factors are needed to show the spatial patterns of Rockford's social geography.

The Social Areas Figure 3, 4, and 5 show the spatial distribution of scores for census tracts for each of the three rotated factors.

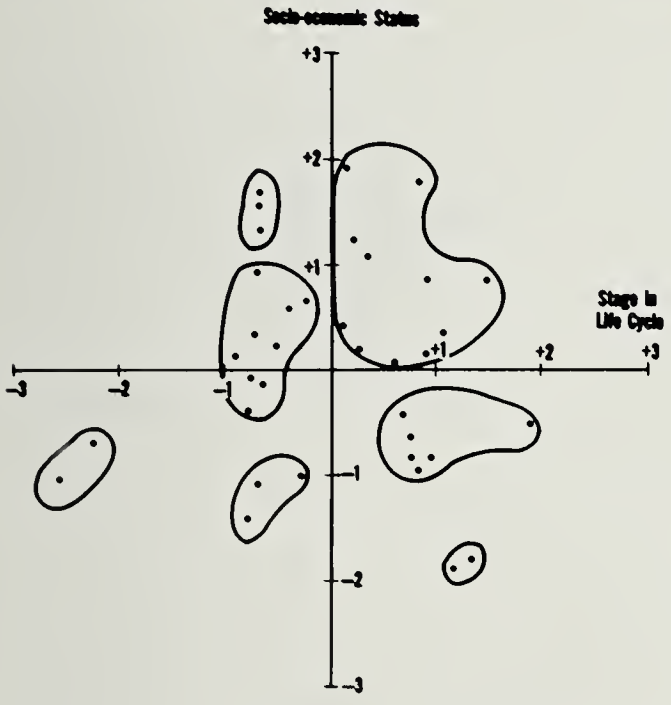

Figure 6. SES vs. LC Scores

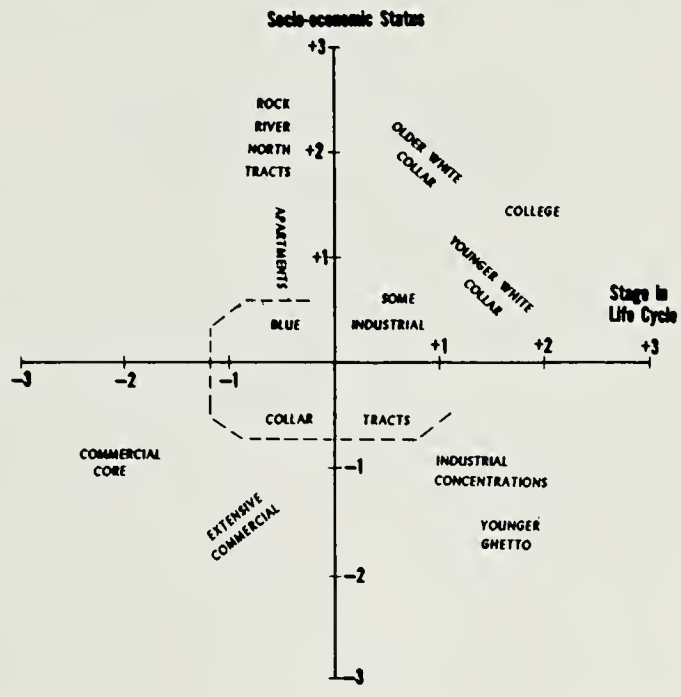

Figure 7. Interpretation of Figure 6 clusters

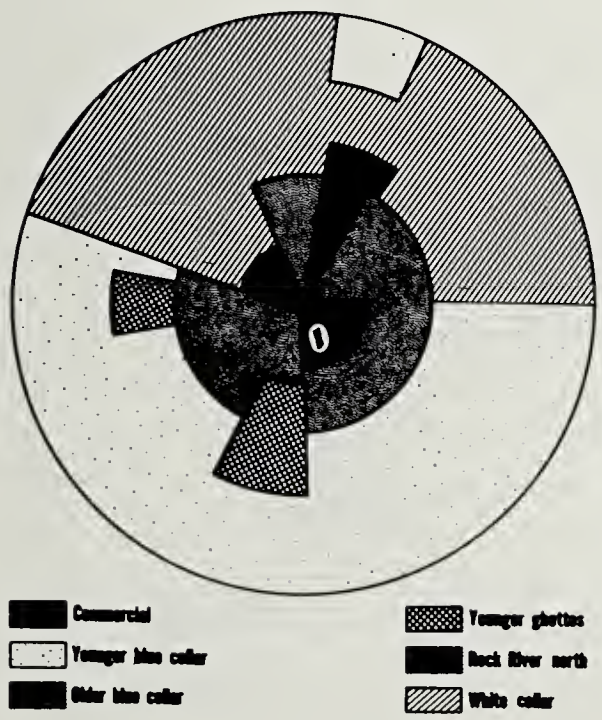

Figure 8. Schematic Diagram of Rockford's Social Areas

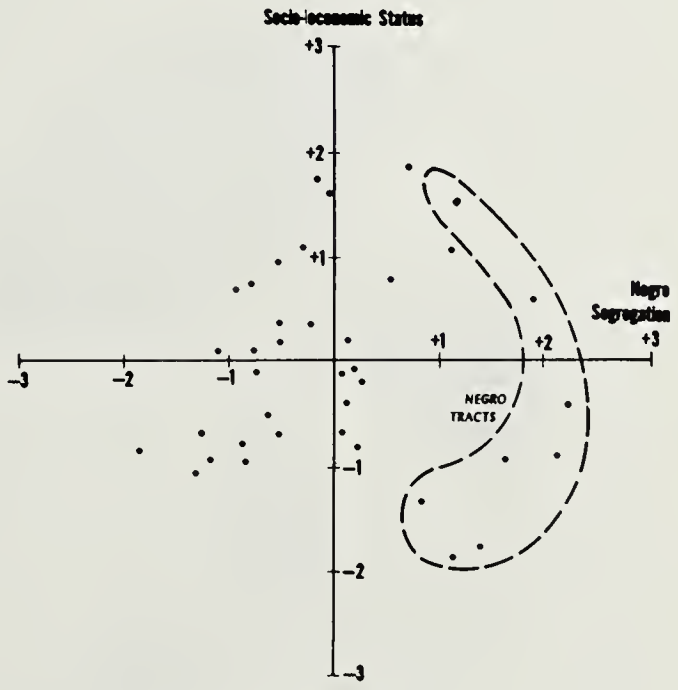

Figure 9. SES v s. NS Scores

PLATE II.

The Factor I (SES) patterns clearly show alternating high and low status sectors radiating outward from the city center. The highest SES sector is to the east, and the largest concentration of low status is in the south. Another low SES area is in the west.

The Factor II (LC) patterns of Figure 4 show the marked concentric

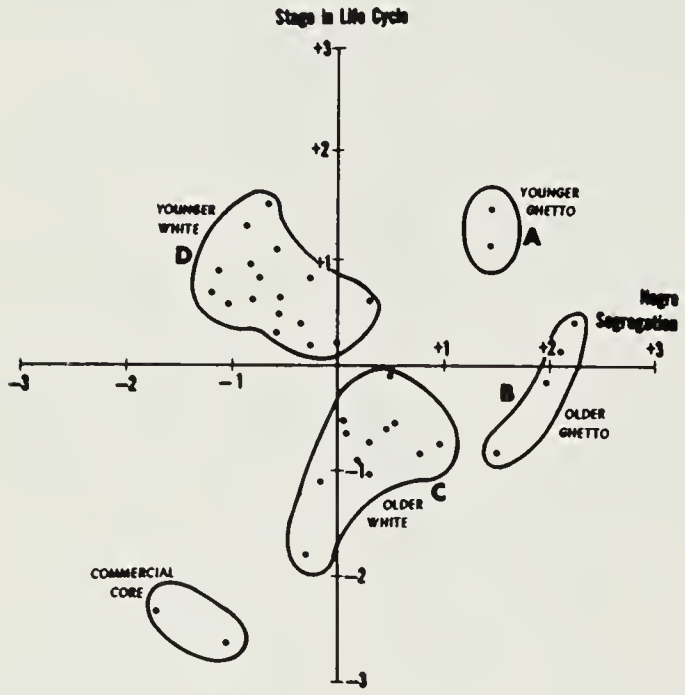

Figure 10. LC vs. NS Scores

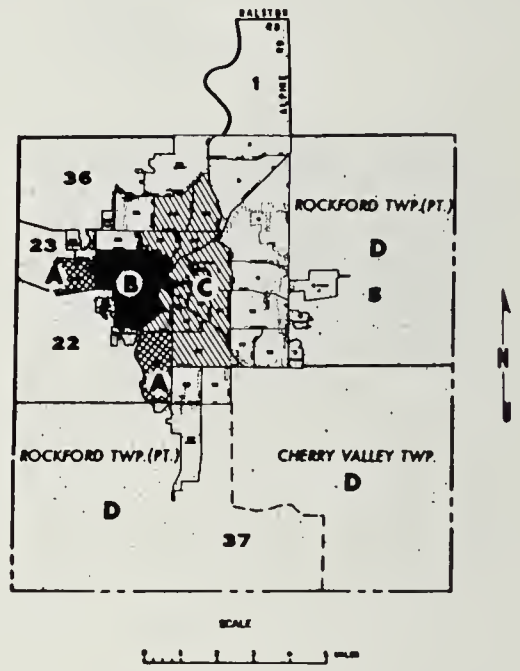

Figure 11. The Spatial Interpretation of Figure 10.

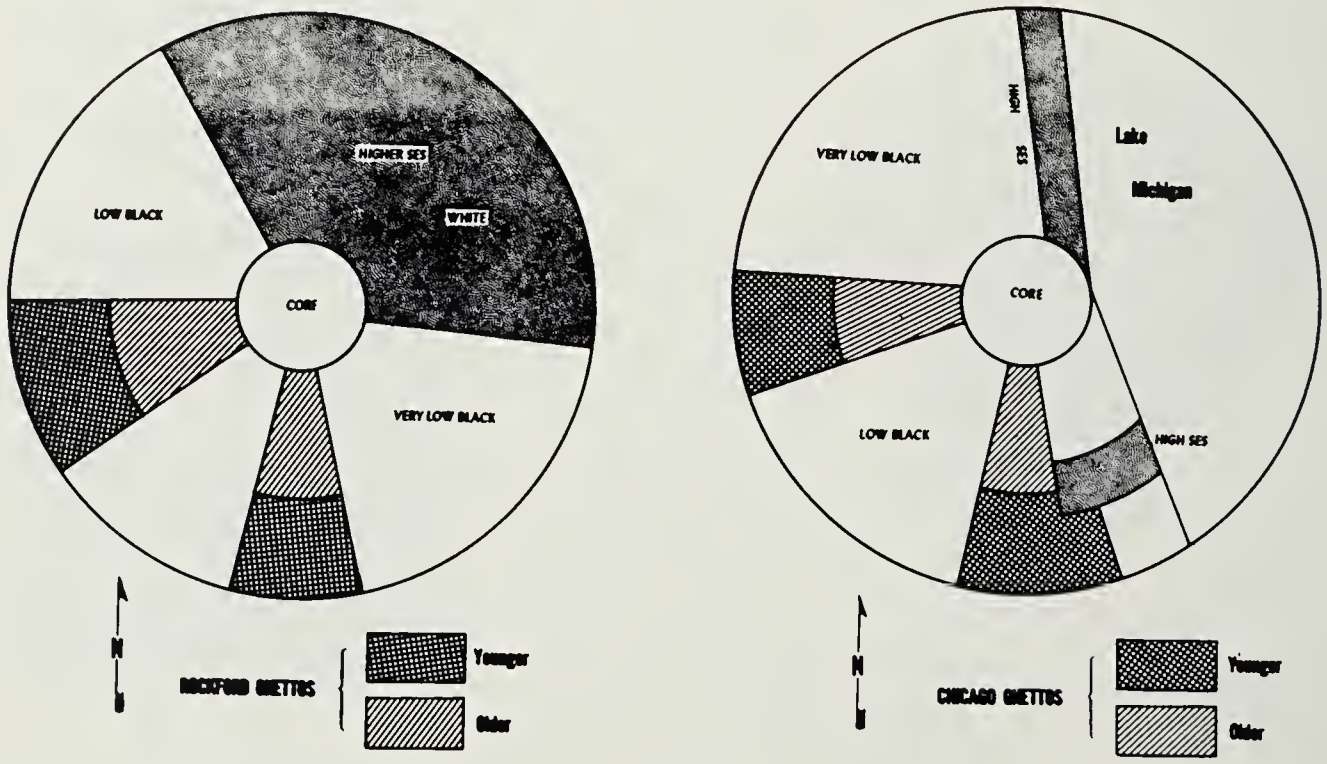

Figure 12. A Comparison of the Rockford and Chicago Ghettos

PLATE III.

ring of more youthful families completely surrounding the older, more central parts of Rockford. Other patterns are more or less concentric; regardless of either social status or ethnicity.

Ethnic concentrations stand out in the Figure 5, Factor III (NS), pattern. Ghetto concentrations appear to radiate in tight sectors outward from the commercial core. The areas with a propensity toward Negro concentrations appear different in terms of age structure. There are clusters of both younger families and older couples. However, nearly all of the Black tracts are in lower status areas. Additional comparisons clear up the above notions.

The next sequence of illustrations, Figure 6, 7, and 8, shows how tracts behave with respect to the first two factors considered similarly. Figure 6 shows groups of census tracts socially similar according to these two measures of SES and LC. Figure 7 shows how the Figure 6 groupings fit in with other functional areas of the city. Rockford College is located in the younger, high SES white collar area. There are some older high SES tracts along the northern Rock River area. Most blue collar areas appear to be older and of lower SES. In addition, blue collar tracts appear to occur adjacent to commercial and industrial areas. The younger ghetto tracts stand out clearly. Figure 8 shows the social mosaic in spatial terms.

Negro Segregation The above discussion indicates the existence of differentiated ghettos in Rockford. Figure 9 plot shows a fairly wide SES range for tracts with higher NS scores. Figure 10 integrates LC and NS scores for tracts further, and five clear clusters form. These are the older and younger white areas, the older and younger black areas, and the CBD. Figure 11 shows the spatial distribution of the integrated LC/NS scores. Thus, in addition to ghettoization we see a range of social status and life cycle amongst Negro tracts. This pattern is not unlike Spear's (1967) examination of the development of Chicago's ghetto areas.

A comparison of the ghettos of Chicago and Rockford shows the striking similarities of the central portion of both cities. This comparison is shown schematically in Figure 12. The scale differential is, of course, great. Coincidentally, ghetto sectors of both cities take the same cardinal directions. More significant is the fact that the ghettos can be differentiated between older and younger population spatially. The younger population groups are located farther away from the central core than the older populations. In both cities high status areas representing the converse of the ghettos are located in northerly sectors.

Conclusions

The data for this study are nearly ten years old, and many changes have taken place. The Rockford SMSA is larger now, and new industrial establishments have located in the area. However, certain principles of neighborhood growth which have been developed from the Chicago and other models (see Hoyt, 1939) dictate that patterns which have been somewhat cemented should continue.

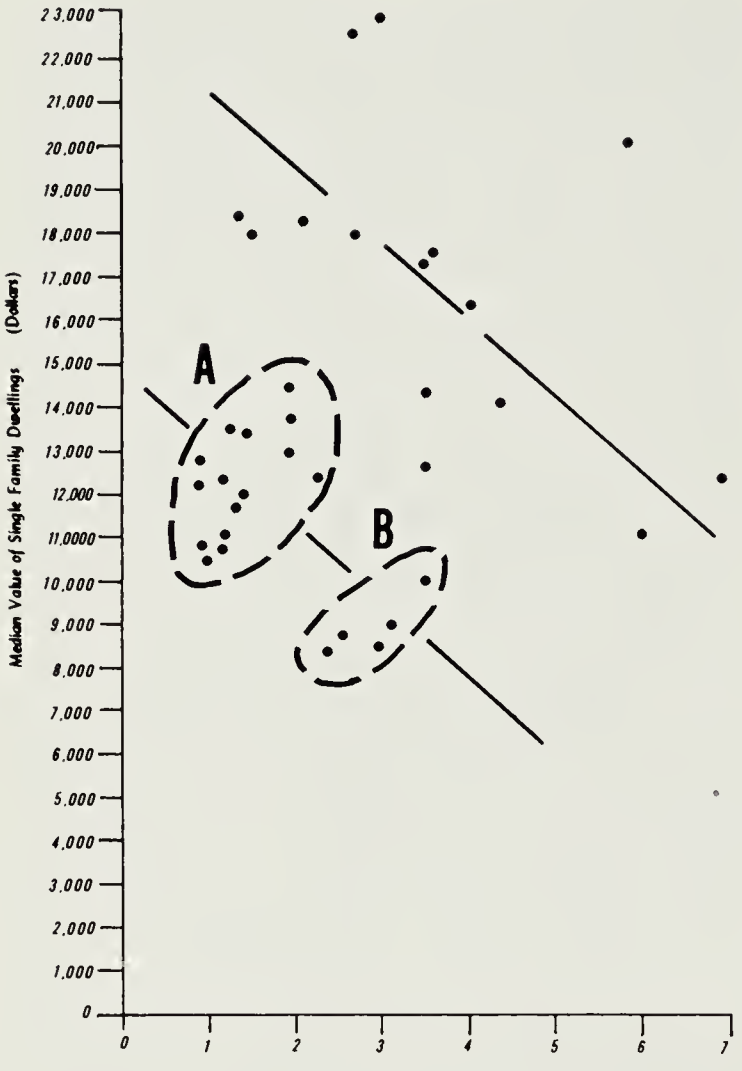

Figure 13. Land Values Compared to Distance from City Center

One way to predict the future growth of Rockford's social areas is to examine the city's land values with respect to distance from the city center. Figure 13 shows several patterns for Rockford. In general, there is a dual system of land values. There is a tendency for high status areas to maintain higher land values than other sectors even with increased distance from the city center. Two lower clusters show (A) the older blue collar tracts and (B) the younger ghetto and younger blue collar tracts as the lower part of the dual system. According to Hoyt and Hurd, similar patterns have become fixed in many other American cities.

Acknowledgments

This paper was prepared as a part of a University of Chicago urban studies project which examined the ecological structure of a wide variety of American cities using factorial methods. The project was directed by Professor Brian J. L. Berry. I am indebted to Professor Berry for reviewing this manuscript.

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SURVEY ON THE BACTERIOLYSIS BY THERMOPHILIC ACTINOMYCETES

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ABSTRACT. — Several strains of thermophilic actinomycetes are found to lyse the living and heat-killed cells of bacteria. The lytic principles appear to be enzymes in nature.

Aside from producing antibiotics, some species of thermophilic actinomycetes are known to synthesize enzymes lytic to bacteria (Lawrence, 1957; Desai and Dhala, 1967). As this group of microorganisms are found abundantly in soils and solid wastes, their ecological importance is of great concern to the public. Studies made so far showed only heat-killed, Gram-negative bacterial cells are susceptible to enzymatic lyses by this group of micro-organisms and no reports have been made on the lysis of living cells by their enzymes. A recent survey showed some living bacterial cells can also be lysed and the preliminary report is presented here.

In the past two years 37 isolates of thermophilic actinomycetes were screened from hundreds of cultures originally isolated from the soils and solid wastes collected from the western Illinois areas. These isolates showed lytic activities when tested against the heat-killed cells of *Escherichia coli B. Aerobacter aerogenes* ATCC 7256, *Proteus vulgaris* ATCC 9920, *Micrococcus lysodeikticus* ATCC 4698, and *Bacillus megaterium* ATCC 6458. A spectrophotometric method (Desai and Dhala, 1967) was used for the detection of the bacteriolysis by the culture filtrate of these isolates which were prepared by growing the organisms for 72 hours at 55 C under constant shaking (New Brunswick water-bath shaker Model G76, 120 rpm) followed by centrifugation to obtain the culture supernate. Starch -N-Z case broth (Kuo and Hartman, 1966) was used for the growth.

Among 37 isolates, 29 were active against *E. coli*, 13 against *A. aerogenes*, 12 against *P. vulgaris*, 3 against *M. lysodeikticus* and only one against *B. megaterium*. *E. coli* cells were found most susceptible to lysis.

When the live cells of these bacteria were tested for the susceptibility toward the culture filtrates of these 37 isolates, only 7 strains of these thermophilic actinomycetes were found capable of lysing these bacterial cells except *B. megaterium*. Two isolates were effective against *M. lysodeikticus*, two against *E. coli* and *A. aerogenes* and one against *P. vulgaris*. *M. Lysodeikticus* and *E. coli* were readily lysed by the culture filtrates within 48 hours of incubation.

When the culture filtrates of these actinomycetes were used as the sources of lytic enzymes, erroneous results sometimes occurred due to the

presence of actinomycete pigments. These pigments interfered with the readings on a spectrophotometer. To eliminate this error, the culture filtrate was run through a filter packed with acid-pretreated activated carbon. The culture filtrate thus obtained was colorless and no interference was therefore observed. Use of these colorless culture filtrates from the 7 isolates as the sources of lytic enzymes for the lysis of living bacterial cells showed two isolates effective against *M. lysodeikticus,* two against *E. coli,* one against each *A. aerogenes* and *P. vulgaris.* It seemed that during the filtration of culture supernates through active carbons some lytic enzymes had been adsorbed.

As bacteriolysis might result from autolysis or through the actions of antibiotics produced by the actinomycetes rather than by lytic enzymes, experiments were then carried out by the use of bacterial cell walls. The walls were prepared by sonication followed by thorough washing in the saline (0.83% NaC1). When these walls were suspended in the culture filtrates and incubated at 55C for 48 hours, lyses occurred in all the cell walls prepared. *M. lysodeikticus* wall was most susceptible to lysis in that complete dissolution of the wall occurred within 12 hours after incubation. *E. coli* was next susceptible and *P. vulgaris* wall least. Spectrophotometric method was again used for the determination of lysis. These experiments showed that the lytic principles in the culture filtrates were enzymes, and not antibiotics nor through autolysis.

The enzymatic nature of the lytic principles were further supported by the facts that the lytic activity of the filtrates was lost by heating them at 100 C for three minutes or at 80 C for twenty minutes. In some culture filtrates where heat-killed cells were lysed the lytic principles seemed to be proteinases as there was linear relationship between the lysis of the cells and the hydrolysis of such proteins as casein, albumin, and gelatin. Loss of proteinase activity in the culture filtrate also resulted in the loss of lytic activity.

The lytic enzyme responsible for the lysis of *M. lysodeikticus* cells seemed to be lysozyme in nature since the main component of the cell wall of this organism is murein and organism has been known to be very susceptible to lysozyme. (Salton, 1964) Mureins are found in every bacterial cell wall in different quantities and it is highly possible that the same type of enzymes are also responsible for the wall dissolution in other species of bacteria used in the experiments. In the lysis of Gram negative bacterial cells, probably both the lysozymes and proteinases participate. Studies on the purification and characterization of these lytic enzymes are in progress.

It is hoped that the taxonomic status of these bacteriolytic thermophilic actinomycetes will be clarified in the near future. Due to the flux in the taxonomy of thermophilic actinomycetes at the present time, there are difficulties to classify these 37 isolates down to generic level. At present, 9 isolates are found to be *Thermoactinonomyces,* three in *Thermopolyspora* and two in *Ther-*

momonospora. No thermophilic *Streptomyces* species has yet been found to produce lytic enzymes.

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A STUDY OF GREAT-HEADED GARLIC IN ILLINOIS

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ABSTRACT. — Unusually large-bulbed *Allium* specimens, grown as a condiment in southern Illinois, were cloned, cytologically investigated, and found to belong to the sterile hexaploid ($2n = 48$) race of the great-headed garlic, *Allium ampeloprasum* L. var. *ampeloprasum*. Chromosomes and specimens are illustrated. Gross morphological data on clonal hexaploids, and from previous and newly reported collections of this "garlic", are tabulated.

Unusually large garlic-like *Allium* bulbs, grown in gardens for use as a condiment by people of Italian descent in Jackson Co., Illinois, were called to our attention in 1964. Several of these "giant" bulbs were obtained and cultivated as clones in Carbondale, outdoors and in a greenhouse, in order to have material to enable us to investigate the cytology and identity of the plants.

Cytological investigations comparing normal and colchicine-treated (conc. 0.05%) root tips of *Allium sativum* and of the "giant" taxon were made. In addition, smear preparations from anthers and paraffin-embedded, sectioned material from ovules of the "giant" taxon were used. Farmer, Navashin, Allen-Bouin, Zenker, and LaCour 2BE solutions were used as fixatives. Aceto-carmine, acetic-orcein, and Feulgen's squash technique were used as rapid stains. Paraffin-embedded material sectioned at 10 microns was stained by the following methods: Feulgen-light green, safranin-fast green, PAS-hematoxylin-picric acid, Fleming's triple stain, and LaCour's crystal violet.

In contrast to the 16 chromosomes counted in the diploid *Allium sativum*, 48 chromosomes were found in our hexaploid "giant" taxon (Figs. 1 and 2). This places the latter

FIGURE 1. 48 chromosomes at C. metaphase. Treated with 0.05% colchicine for 12 hours.

Upper arrow: One member of the homologous pair of chromosomes with a subterminal centromere.

Lower arrow: One member of the homologous pair of chromosomes bearing a constriction on the short arm. See also insert marked by double arrow.

FIGURE 2. 96 chromatids at C. anaphase. Cells treated with 0.05% colchicine for 12 hours.

FIGURE 3. C. metaphase showing approximately 60 chromosomes. Cells treated with 0.05% colchicine for 12 hours.

FIGURE 4. Metaphase and anaphase with slanted equatorial plates.

FIGURE 5. Metaphase with diagonally located equatorial plate. Note the crowded condition of the chromosomes and the narrow nucleoplasm ratio.

PLATE I.

taxon in section Porrum, (basic number x = 8) of the *Allium* genus. Occasionally, however, cells were found with chromosome numbers either higher or lower than 48, such as 34, 39, 40, 60, 62, and circa 74 (Fig. 3). This chromosomal instability is apparently caused by a noticeably lowered nucleus-cytoplasm ratio found in the cells of the hexaploid taxon. In fact, about 28% of the mitotic prometa- or metaphase configurations exhibited a slightly slanted or sometimes diagonally-positioned equatorial plate (Fig. 4). This alteration of mitotic geometry elongates the equatorial plate, and assures better accommodation during the lining up of the centromeres of the chromosomes. In relatively narrow cells, (Fig. 5) however, the long axis of the spindle becomes shorter, which in turn hinders the complete separation of the sister chromatids from each other. Incomplete separation of such chromatids was frequently observed. In some cells, this condition leads to the separation of chromatids into 3 or 4 groups which were found lying in tandem position in the narrow, long cells.

As expected, the size of the nuclei of our hexaploid taxon was larger, and measured in Feulgen-stained material an average of 1552.5 cubic microns in volume, while the diploid measured only 597.1 cubic microns. Both of these averages were calculated from the measurement of 400 nuclei. Similarly, the size of the nucleoli increased noticeably. However, the average number of nucleoli per nucleus showed only a slight increase yielding an average of 2.34 in the hexaploid and 2.19 in the diploid. Out of the 48 chromosomes, 46 carried median or submedian centromeres, and 2 carried subterminal ones (Fig. 1, upper arrow). Two chromosomes which had submedian centromeres and a constriction on the short arm (Fig. 1, single and double arrow) were considered as nucleolar chromosomes. The presence of a third single chromosome with a median centromere bearing 2 constrictions on the same arm indicates that this taxon is karyotypically heterozygous. None of the chromosomes had a typical satellite. The low average number of nucleoli found in the hexaploid taxon is related to the fact that only 3 constrictions bearing chromosomes were present in this karyotype.

It is pertinent to note that a competition between nucleolar chromosomes discovered by Navashin (1934) in *Crepis* hybrids might eliminate not only the function, but also the nucleolar constriction of the functionally suppressed chromosomes. In our taxon a similar phenomenon may account for the low number of chromosomes carrying a nucleolar organizer.

Early degeneration of the sporogonial tissue, causing total sterility, was observed in the anthers. Abnormal pollen mother cells, heterogeneous in size and shape, were also found but only in a few cases. Apparently, some of the cells of the sporogonial tissue were able to differentiate to that level. However, meiosis was never observed in these abnormal pollen mother cells. Similarly, total sterility caused by early degeneration of the nucellar tissue was found on the female side, although in 4 cases we observed megaspore cells, and in one case an embryo sac-

like complex, containing pycnotic nuclei.

It is interesting to note that a similar large-sized garlic marketed in northwest India as a substitute for the normal garlic was collected by Khoshoo and Sharma (1957), and found to be also hexaploid and totally sterile on both the male and female sides. Khoshoo, Atal, and Sharma (1960) identified this taxon as *Allium ampeloprasum* Linn. The heterozygous karyotype contained only median and submedian centromeres, among which were 3 pairs of homologous chromosomes and 6 single chromosomes which carried nucleolar or secondary constrictions.

Ved Brat (1964) established 6 different types of nucleolar chromosomes in *Allium* genus. The section Porrum is characterized by two types, "Sativum" and "Scorodoprasum." Both types have a "median-submedian" centromere and one constriction, which is proximal in the "Sativum" type and "median-submedian" in the "Scodoprasum." In contrast to the findings of Ved Brat and Khoshoo, Atal, and Sharma, our taxon had a pair of chromosomes with a subterminal centromere (Fig. 1), only one pair of nucleolar chromosomes, plus a single chromosome with 2 secondary constrictions.

Male sterility is a well-known phenomenon in *Allium,* and the development of sterile anthers is frequently associated with the presence of viviparous bulbils which occur mixed with the flowers in the umbel. However, in our hexaploids, bulbils were never found.

The large size of the vegetative

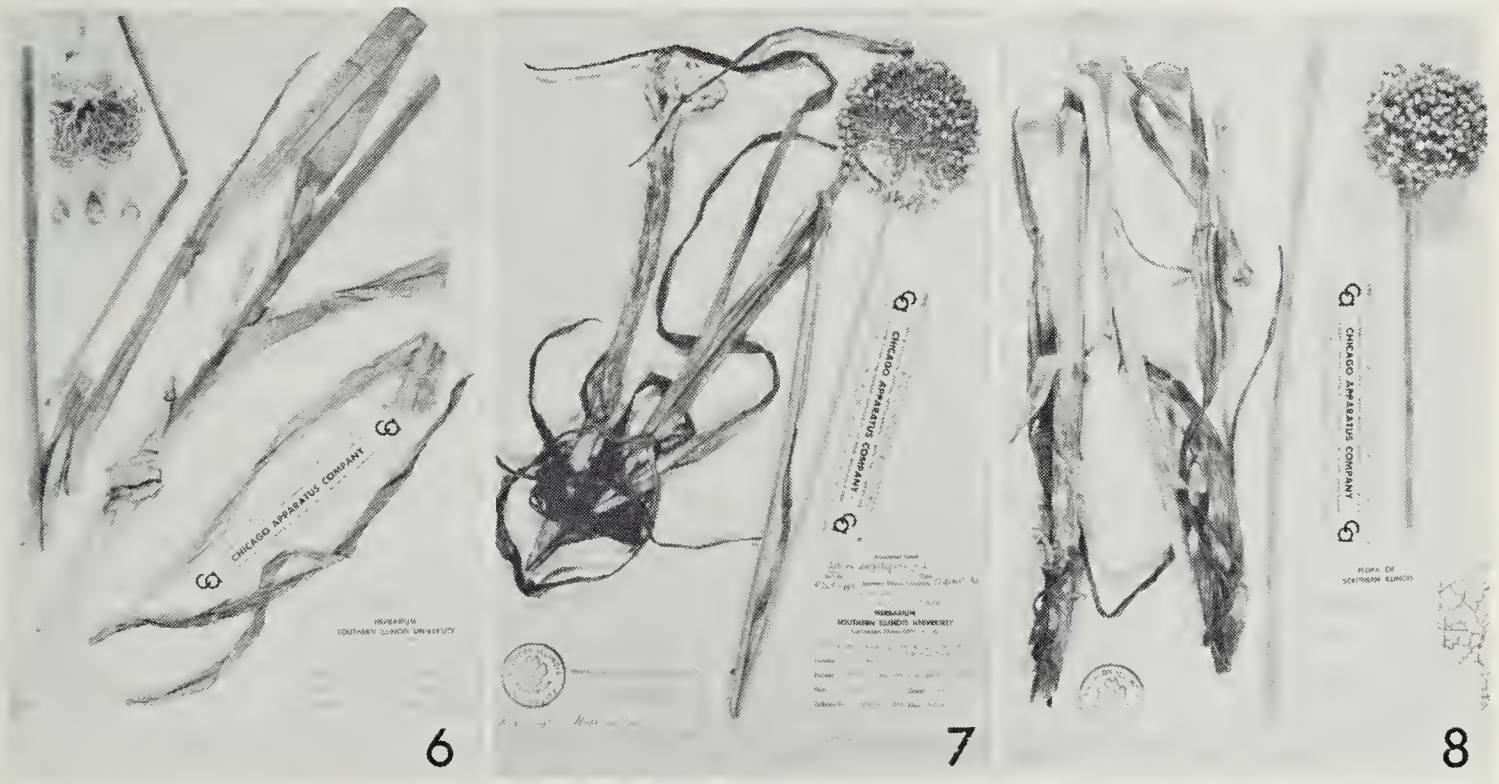

PLATE II.

FIGURE 6. Specimen from a Carbondale-cultivated clone of *A. ampeloprasum* L. var. *ampeloprasum* known to be hexaploid and sterile. The stem is 12.3 dm tall; note large and small bulblets comprising bulb.

FIGURE 7. Specimen of *A. ampeloprasum* L. var. *ampeloprasum* which was growing spontaneously in Pope County, Illinois. The stem is 12.2 dm tall.

FIGURE 8. Specimen of *A. ampeloprasum* L. var. *ampeloprasum* cultivated in Pope County, Illinois. The stem is 15.4 dm tall.

TABLE 1. — Vegetative Characters of *A. ampeloprasum* L. var. *ampeloprasum*.

Specimen	Bulb Diameter (cm)	No. of Cloves in Bulb	Clove Length (cm)	Clove Diameter (cm)	Stem Height (dm)	No. of Leaves*	Leaf Length (dm)	Leaf Width (cm)
DeFilipps 1 (SIU)	4.5	8 (5 large & 3 small)	2.2-3.0 large; 1.2-1.6 small	1.5-3.0 large; 1.2-1.6 small	12.3	5	4.4-4.8	2.5-2.6
DeFilipps 2 (SIU)	5.5	4	2.6-4.0	1.6-3.0	13.5	7	3.9-4.1	1.2-1.7
Hopkins 1208 (SIU)	5.0	3 (incomplete bulb)	3.0-3.3	1.7-2.0	12.2	6	3.9-4.5	1.4-1.5
Wiley s.n. (SIU)	5.0	4	4.0-5.0	2.7-3.5	15.4	5	5.1	2.0-2.3
Fuller & Fisher 115 (F)	—	—	—	—	6.0 (incomplete specimen)	3	4.3	1.6
Khoshoo, Atal & Sharma's hexaploids (1960)	5.0-6.5	1; 4-6	3.0-4.5	—	8.9-9.0	—	3.0-3.5	1.5-1.7

* All specimens have flat leaves with scabrous margins and scabrous keel.

organs, the sterile flowers, and the absence of inflorescence bulbils make our material referable to the great-headed garlic, *Allium ampeloprasum* L. var. *ampeloprasum* (Fig. 6). This taxon grows wild in Europe, Southwest Asia, and North Africa (Hegi, 1909). It has been introduced into the United States, where it is cultivated for use as a condiment and as an ornamental (due to its large overall size), and escapes and persists near dwellings. It has been recorded from several states, including Tennessee (James, 1956), North and South Carolina (Ahles, Bell & Radford, 1958), Kentucky (Browne, 1963), Mississippi (Pullen, 1967), Alabama (Jones, 1968), and Illinois (Browne, 1963).

The basis for the previous Illinois record is *Fuller & Fisher 115 (F)*, collected in wet fields in the Union County State Forest in 1940. In 1968 this area was searched by the junior author and Mr. A. Skorepa, but no specimens were located. Two more recent Illinois collections are extant: *Hopkins 1208* (SIU), near an old home ¼ mile north of Lusk Creek Gorge, Pope Co. (Fig. 7), and *Wiley s.n.* (SIU), cultivated in a garden in Harrisburg, Pope Co. (Fig. 8). Details of the gross morphology of these two collections and from two plants from our hexaploid

TABLE 2. — Floral Characters of *A. ampeloprasum* L. var. *ampeloprasum*.

Specimen	Umbel Diameter (cm)	Pedicel Length (cm)	Tepal* Length (mm)	Pistil Length (mm)	Style Length (mm)	Filament Condition	Seeds
DeFilipps 1 (SIU)	9	4.5	4.5	4.0-4.5	2.5-3.0	inner 3 with appendages	none
DeFilipps 2 (SIU)	8	3.5-4.5	4.5	4.0-4.5	2.5-3.0	inner 3 with appendages	none
Hopkins 1208 (SIU)	8.5	4.0	4.5-5.5	4.0-4.5	3.5-4.0	inner 3 & outer 3 with appendages	few, shriveled
Wiley s.n. (SIU)	8.5	3.0-3.5	4.0	4.0	2.0-3.0	inner 3 with appendages	few, shriveled
Fuller & Fisher 115 (F)	7.5	3.0	4.2-4.5	3.5-4.0	3.0	outer 3 with appendages	none
Khoshoo, Atal & Sharma's hexaploids (1960)	—	—	—	—	—	inner 3 & outer 3 with appendages	none

* All specimens have outer 3 tepals verrucose-scabrate; inner 3 often not or weakly verrucose-scabrate.

clone, *DeFilipps 1* (SIU) (Fig. 6), and *DeFilipps 2* (SIU) are given in Tables 1 and 2.

Two current studies of cultivated *Alliums* differ in their circumscription of *A. ampeloprasum*. Jones & Mann (1963) prefer to treat this species as composed of three entities: great-headed garlic, leek, and kurrat, indicating that they were probably derived from the same wild parent. Millan (1952) separates the kurrat (*A. kurrat,* Schweinfurth ap. Krause) from *A. ampeloprasum,* the latter including the great-headed garlic (*A. ampeloprasum* L. var. *ampeloprasum*) and the leek (*A. ampeloprasum* L. var. *porrum* (L.) Gay). These taxa may be differentiated, according to Millan (1952), as follows:

1. Umbel with bulbils.
   2. Bulbs without cloves (bulblets) .....*A. kurrat*
   2. Bulbs with cloves (bulblets) .....*A. sativum*
1. Umbel without bulbils.
   3. Plants with bulbs; bulbs with large and small cloves; flowers sterile ....*A. ampeloprasum* var. *ampeloprasum*
   3. Plants with an enlarged

base, but without bulbs; flowers fertile ....*A. ampeloprasum* var. *porrum*.

Previous cytological studies have shown that *A. ampeloprasum* var. *ampeloprasum* (sensu Millan, 1952) exists in three forms or races: Diploid with 2n = 16 (Renzoni, 1964), tetraploid with 2n = 32 (Maude, 1940; LaCour, 1945; Ved Brat, 1965), and hexaploid with 2n = 48 (Khoshoo & Sharma, 1957; Khoshoo, Atal & Sharma, 1960; Ved Brat, 1965.) In the reported hexaploids the source of investigated material was India, and the two former articles (1957, 1960) reported total sterility of flowers, whereas the latter reported vivipary. Ved Brat (1965) noted tetraploid *A. ampeloprasum* as being a sexual outbreeder and hexaploids as viviparous asexually.

According to our study of living specimens, this taxon in Illinois is hexaploid and sterile, and thus cannot breed sexually. However, no bulbils were found in any inflorescences, so reproduction by vivipary is excluded. In Illinois this variety is reproduced vegetatively from bulblets from the underground bulb, which is usually compound. Simple-bulbed plants have been observed in our study, as well as by Khoshoo & Sharma (1957), Khoshoo, Atal & Sharma (1960), and Millan (1952).

Khoshoo, Atal & Sharma (1960) noted that *A. ampeloprasum* var. *ampeloprasum* is karyotypically heterozygous. The heterozygosity, which may have arisen either after its origin or more probably as a result of hybridization between karyotypically dissimilar forms preceding the institution of polyploidy, is maintained by the vegetative reproduction, which has been discussed by Galil (1965, 1966). At present it is not possible to comment on the origin of the hexaploid race and/or its relationship to other races until detailed studies of all the karyotypes concerned are available, a conclusion also reached by Khoshoo, Atal & Sharma (1960).

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SEASONAL VARIATION IN THE FATTY ACID COMPOSITION OF THE WOODLOUSE, *TRACHEONISCUS RATHKEI* BRANDT

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ABSTRACT. — The fatty acid composition of *Tracheoniscus rathkei* was determined monthly from March through January. The predominant saturated acid was palmitic acid (16:0), and the predominant unsaturated acids were palmitoleic acid (16:1), oleic acid (18:1), and linoleic acid (18:2). Lipid unsaturation increased during midwinter due to increases in the proportions of arachidonic acid (20:4) and docosahexaenoic acid (22:6).

Among the various groups of arthropods, the analysis of body lipids has received the most attention in the insects (Fast, 1964; Young, 1967). Even within this class, however, little is known of seasonal variations within natural populations. Preliminary reports on changes in fatty acid composition during hibernation have appeared for a mosquito (Buffington and Zar, 1968) and a beetle (Zar, 1968). This paper has extended these studies to include seasonal changes in the woodlouse *Tracheoniscus rathkei* Brandt (Isopoda: Oniscidae).

MATERIALS AND METHODS

Specimens of *Tracheoniscus rathkei* were collected (about 120 per month) in Trelease Woods, a deciduous forest about 10 miles northeast of Urbana, Illinois. The animals generally were found under leaf litter and logs, and under the bark of logs. During the winter, however, from November through January, they were found only within log crevices, on the undersurface of logs, or in the soil. The methods utilized in the preparation of the woodlice for analysis were as described by Buffington and Zar (1968), as were the fatty acid determinations by gas-liquid chromatography. Chromatograms were run as long as the known retention time for methyl docosahexaenoate.

RESULTS AND DISCUSSION

The mean ($\pm$ SE) water content and caloric value of the animals were 66.1 $\pm$ 4.7 percent and 3521 $\pm$ 2 g-cal/gm, respectively. Table 1 presents the results of the fatty acid analysis. The iodine values were calculated for each fatty acid mixture from the fatty acid composition. The results obtained indicate that the fatty acid composition of *T. rathkei* is unlike that of insects or arachnids that have been studied (Fast, 1964; Young, 1967).

In some insects (Fraenkel and Hopf, 1940; Munson, 1953; Harwood and Takata, 1965) and aquatic crustaceans (Lewis, 1962; Farkas

TABLE 1. — Monthly fatty acid analysis of *Tracheoniscus rathkei* from 14 March 1966 through 22 January, 1967. Table values are percents (by weight).

	14 March	15 April	15 May	15 June	15 July	16 August	15 Sept.	15 Oct.+15 Nov.	14 Dec.	22 Jan.
Fatty acids*										
12:0	2.2	1.3	1.8	1.1	2.1	1.6	2.4	0.8	2.5	1.1
14:0	0.5	0.6	0.4	0.6	1.2	1.0	0.7	1.0	0.8	0.8
16:0	12.6	15.3	12.8	15.7	15.6	17.8	16.6	14.0	12.4	12.1
16:1	14.5	14.7	9.6	9.8	8.6	10.4	10.4	11.0	9.0	7.2
18:0	1.5	2.2	2.8	3.0	3.8	3.3	2.6	2.0	2.4	2.3
18:1	28.7	36.8	30.0	31.6	22.5	26.5	26.4	29.4	33.4	29.1
18:2	15.4	19.6	17.2	18.4	18.9	16.8	19.9	22.0	21.7	19.2
18:3	2.1	1.1	3.9	3.7	3.7	4.1	2.9	3.9	4.9	3.1
20:4	7.2	1.5	4.0	3.0	14.0	6.9	8.1	2.9	4.4	10.2
22:6	7.2	0.1	0.4	1.4	4.4	0.0	0.0	2.5	<0.1	6.9
other†	8.0	5.7	16.9	11.7	6.3	11.4	10.0	10.4	8.4	8.1
Iodine value of fatty acids	143	100	106	113	148	111	115	120	116	152
Body lipids (% of dry wt.)	9.3	11.2	9.8	11.4	8.6	9.3	9.4	10.8	8.4	22.7
Body wt., mg (Mean, dry)	9.2	8.5	8.4	15.1	9.8	11.5	9.4	10.2	10.4	8.0

* Carbon chain length: number of double bonds.
† Includes 10 minor components, tentatively identified as 12:1, 14:1, 14:2, 16:2, 20:1, 20:2, 20:3, 22:0, 15:0, and 17:0.

and Herodek, 1964), a positive correlation between lipid saturation and ambient temperature has been shown. Likewise, *T. rathkei* exhibited its greatest degree of fat unsaturation in January, the month with the coldest environmental temperature. This increase in total unsaturation (as indicated by the iodine value of the fatty acid mixture) was due mainly to the relatively increased amounts of arachidonic acid (20:4) and docosahexaenoic acid (22:6). The increase in 20:4 is not as striking, however, as that found in the same January in a ladybird beetle (Zar, 1968); instead, the woodlouse exhibits a large increase in 22:6, a fatty acid not even detected in the beetle. If the 20:4 and 22:6 are membrane rather than triglyceride fatty acids, their increase may be related to increased cold tolerance in a manner similar to that in plants, in which higher mitochondrial membrane lipid unsaturation is more chill-resistant (Lyons *et al.*, 1964; Lyons and Asmundson, 1965).

According to Hatchett (1947) and Judd (1963), *T. rathkei* mates in April and May and the young emerge 35-42 (mean of 39) days later, there generally being but one brood per year. The 15 June collection in the present study consisted largely of gravid females. This accounts for the high June weight of the animals, but it is of interest that July, after the young had emerged, was a period of high lipid unsaturation.

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ADDITIONAL OBSERVATIONS ON SOME SOUTHERN ILLINOIS MAMMALS

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ABSTRACT. — A descriptive account reflecting additional records of occurrence in southern Illinois for 10 mammals is presented.

Layne (1958) contributed significantly to the updating of information on the distribution and occurrence of southern Illinois mammals. Prior to and in the 12 years since his survey, there have been accumulated additional data that warrant documentation. Our collecting and observing were limited generally to the southern 34 counties of Illinois, collectively identified as the Southern Zone by the Illinois Department of Conservation. The collections of the Zoology Department and Cooperative Wildlife Research Laboratory of Southern Illinois University contain specimens reported on.

SPECIES ACCOUNTS

Terminology employed reflects that of Hoffmeister and Mohr (1957), except for the golden mouse in which case preference is given to Hooper (1958). The use of this terminology is not without recognition of conditions for intergradation in this portion of Illinois as suggested by Layne (1958).

Sorex longirostris longirostris Bachman. Southeastern shrew. — As noted by Hoffmeister and Mohr (1957) this shrew is rare, having been recorded only for Alexander, Coles, Fayette and Johnson counties. Two specimens in our collection extend the occurrence of this shrew. E. W. Pearson trapped an adult female, February 1959, in Union County. Jack White found an immature along a roadway, June 1968, about 4 miles east of Eddyville in Pope County. This specimen appeared to have been dropped by a predator, probably a fox, and was in such state of decay that sex and measurements could not be established.

Taxidea taxus taxus (Schreber). Badger. — Previous reports suggest the badger to be common locally in the northern half of Illinois and known as far south as Fulton and Douglas counties (Hoffmeister and Mohr 1957). According to Robert Kennicott (Cory 1912) as late as 1828 it had been seen as far south as the Kaskaskia River; but, the specific part of this drainage was not identified.

In May 1965, a male was shot in an alfalfa field about 1 mile northwest of Roots in Randolph County by Vernon Hentis of Modoc; the specimen was mounted but the carcass was made available to our laboratory. Without the skin and head, it weighed 5 lb 2 oz; the reproductive tract indicated an immature ani-

mal. The digestive tract contained remains of a fox squirrel.

A second animal, a male and probably adult, was found as a road kill and buried by personnel of the Illinois State Highway Department in September 1965. Its identity was verified by our Laboratory after extraction. This location was in Washington County, 6 miles south of Nashville, approximately 35 air miles east and latitudinally about 15 miles north of where the animal was shot in Randolph County.

Both locations have been subjected to intensive intertilled cropping with only limited acreages devoted to meadows, pasture and small grain. Idle lands and wooded areas represent around 10-20 percent of the land use pattern.

Canis latrans frustror Woodhouse. Coyote. — During the past 19 years our Laboratory has examined over 20 specimens of so-called "wolves." Of these, nine were probably coyotes, the remainder being a variety of feral dogs; five coyotes were available for study.

A 23 lb adult female, shot 3 miles west of DuQuoin in Perry County, showed seven placental scars and evidence of cottontail, meadow vole and chicken in the digestive tract; a 26 lb 13 oz adult male from the bottomlands of the Big Muddy River, 2 miles north of Blairsville in Williamson County, contained only blades of grass in its digestive tract; a 22 lb 5 oz adult female from 2½ miles northeast of Salem in Marion County, yielded six placental scars and traces of prairie chicken, cottontail and blades of grass in the digestive tract; a 23 lb 2 oz male taken 2 miles northwest of Carbondale in Jackson County, contained no food items; and, the digestive tract of a 24 lb 4 oz male, shot 5 miles southeast of Vienna in Johnson County, yielded cottontail and meadow vole, the latter in great quantity. All animals were free of internal worm parasites.

It seems apparent that many reported coyotes or wolves are simply feral dogs. Five animals examined, resulting from organized "wolf" hunts, were without question domestic animals, usually exhibiting strong German Shepherd characteristics. However, it is believed that coyotes have increased in the last 15-20 years and probably are widely distributed in southern Illinois but still scarce in numbers.

Lynx rufus rufus (Schreber). Bobcat. — This rare and infrequently recorded cat is probably more widely distributed than generally appreciated. Hoffmeister and Mohr (1957:123) suggest that the bobcat "occurs in wooded bottomlands . . . of some of the major rivers in heavily wooded regions of southern and possibly of northwestern Illinois." Layne (1958) commenting on reports indicated reasonably reliable evidence of the bobcat occurring in Alexander, Massac and Perry counties.

Since 1955, two specimens from southern Illinois have been examined. Also, a skin was seen from an animal taken by a trapper near Greenville in Bond County; tracks were observed in snow about 1 mile east of Aldridge in Union County; H. Tate, formerly of Lawrenceville in Lawrence County, reported seeing a bobcat on two (1954 and 1956) occasions about 5 miles north of Law-

renceville along the Wabash River; and, tracks were observed in snow in the wooded bottomland along the Little Muddy River about 1½ miles north of Route 14 just east of Old DuQuoin in Perry County.

A female, shot June 1961, 2 miles southwest of Olive Branch in Alexander County, weighed 15 lb 14 oz; three placental scars were evident and nematodes and cestodes occurred in the digestive tract which contained no food items. A 25 lb 12 oz female, shot December 1961, in the lower end of Dog Tooth Bend in Alexander County, showed four placental scars and its stomach contained much chicken; cestodes were prevalent in the small intestine.

Ursus americanus americanus Pallas. Black bear. — On 6 May 1966 a field trip group from the Department of Recreation and Outdoor Education of Southern Illinois University reported seeing a bear in the southwest corner of Williamson County (SW¼, S16, T10S, R1E); identifiable plaster casts of its tracks were taken. The animal was again seen on 7 May by Mr. and Mrs. Herman L. Batson in the SE¼, S9, T10S, R1E of Williamson County. On 8 May representatives of the Crab Orchard National Wildlife Refuge trailed the animal for about 300 yards. It was next reported near Spring Garden, Jefferson County (S22, T4S, R3E) on 15 May; and, on 17 May, tracks were reported near Ewing, Franklin County (S15, T5S, R3E). On 19 May, hounds were released on the Lowell Davis farm southwest of Mt. Vernon; the chase lasted 1½ hours resulting in the bear being killed near McClelland School, Jefferson County. The site of the kill was about 55 air miles from the location where it was first seen. The specimen, a 305 lb male, is now mounted and on display in the Jefferson County Courthouse, Mt. Vernon. Our Laboratory examined the digestive tract which yielded turtle eggs and a quantity of white clover.

Because the black bear occurs in states adjacent to this region of Illinois, the possibility of an occasional "invader" is conceivable. The possibility of a release or an escapee, however, should not be minimized.

Geomys bursarius illinoensis Komarek & Spencer. Plains pocket gopher. — Two specimens were taken in St. Clair County on 12 March 1965 and another on 29 September 1966; Mohr (1946) previously reported gophers in this county. A female adult was taken at 1901 W. Belle Ave. and a male at 609 N. 43rd. St. of Belleville, in lawns of private homes. The blue grass lawn of the first capture showed seven mounds, the oldest being near the street. The resident across the street reported gopher problems the previous year. An adjacent weedy lot showed no mounds. Approximately 15 mounds were in the lawn where the second capture was made. Although an adjacent resident reported having gophers a few years ago, search of the neighborhood revealed no other mounds. Both sites of capture were about .5 mile from the edge of the city and were separated from one another by about 3 miles.

Although no trapping was conducted, probable gopher mounds were noted in July 1955 in a red clover meadow 2.6 miles south of the intersection of Illinois routes 4 and

13 in Randolph County, and in September 1956 3.1 miles south of Freeburg in the grassy roadside of Illinois Route 13 in St. Clair County. These are the first reports of the pocket gopher for this area since Mohr's (1946) latest observations and collections in 1941. That this gopher did exhibit previous populations in southern Illinois is evidenced by Howell (1910) who reported it for Randolph, Jefferson, Marion and Richland counties. It is apparent that this population, isolated from the more extensive population that is restricted to a two to three county-wide area along the east and south sides of the Illinois River, continues to survive.

Ochrotomys nuttalli aureolus (Audubon and Bachman). Golden mouse. — Hoffmeister and Mohr (1957) stated that this mouse had been taken only in Alexander and Johnson counties; however, Robert Kennicott (Cory 1912) reported two captures at Murphysboro, Jackson County and noted that it was "not very uncommon near Salem," Marion County. Layne (1958) added Union County; Andrews (1963) and Blus (1966) noted fairly sizable populations for Johnson and Union counties, respectively; and, our collections contribute Pope and Perry counties as well as new locations of captures for Jackson and Alexander counties.

These collections suggest that the golden mouse may be more widely distributed than generally appreciated. Almost without exception the recent occurrences of this mouse reflect the presence of catbrier (*Smilax* spp. or grape vine (*Vitus* spp.) entanglements in trees or stands of cane (*Arundinaria gigantea*) either along narrow, upland waterways, or as found in floodplains of major streams. Most populations tend to be small and isolated except where the habitat requirement might be distributed through an extensive area. It is not uncommon to trap only one animal at a given site. Intensive trapping in specific habitats may well result in captures of the golden mouse in many of the southern 34 counties.

Oryzomys palustris palustris (Harlan). Rice rat. — Klimstra and Scott (1956) greatly extended the previously reported range of this mammal in southern Illinois. Subsequent small mammal trapping has added further to our knowledge of the distribution of the rice rat in the Big Muddy River drainage.

Four specimens, taken during February 1960, 2 miles southwest of Cambria, represent the first records for Williamson County. All captures were in or near a drainageway, exhibiting native or domesticated grasses as the principal ground cover. Generally this waterway was about 20 feet wide and it passed through an intertilled-crop area. Sites of capture were approximately 3 miles to the south of the main channel of the Big Muddy River.

Massac County is represented by three specimens trapped in a lowland bromegrass meadow adjacent to U. S. Route 45 .5 mile east of Metropolis, October 1959. Five rats taken in a wet, woods-grass area in the La Rue Swamp, 1 mile east of Aldridge, October 1958, extend their occurrence to the western portion of Union County.

Zapus hudsonius intermedius

Krutzsch. Meadow jumping mouse. — Although Hoffmeister and Mohr (1957) state that the meadow jumping mouse is of state-wide occurrence, records by county in southern Illinois are scarce and incomplete. Over a 16-year period approximately 200,000 trap nights employing the Museum Special Trap in 21 southern Illinois counties yielded 11 specimens, representing only five counties.

Previous reports (Necker and Hatfield 1941; Gersbacher and Swayne 1951; Layne 1958; and Stains 1963) show that the jumping mouse occurs in Perry, Jackson, Union and Franklin counties. Our trapping also produced specimens for Jackson and Union counties but added as new records Alexander, Massac and Pulaski counties. These captures extend the recorded range of this mammal to the southern boundary of the State.

Sylvilagus aquaticus aquaticus (Bachman). Swamp rabbit. — Hoffmeister and Mohr (1957) stated that the swamp rabbit occurs in the southern third of Illinois. However, published records, based upon specimens in collections represent only Alexander, Jackson, Union, Johnson, Williamson and Marion counties (Cory 1912; Necker and Hatfield 1941; Layne 1958). Collecting since Layne's efforts in 1954-55 has yielded specimens representing Perry, Jefferson, Massac and Wayne counties. Also, what are believed positive verifications have been recorded for Gallatin, Wabash, Lawrence, Washington, Bond and Calhoun counties. Further, a former member of the Illinois Department of Conservation reported the harvest of "5 and 6 lb" rabbits near Quincy in Adams County.

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OBSERVATIONS ON THE SUSCEPTIBILITY AND DEGREE OF INJURY TO CRABAPPLES FROM APPLE SCAB

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ABSTRACT. — In 1966, 1967, and 1968 the crabapple collection at the Morton Arboretum was surveyed for the occurrence of apple scab. Thirty-one taxa were found free of infection during this period.

A number of other reports have been made on the susceptibility of ornamental crabapples, *Malus* sp., to apple scab, *Venturia inaequalis* (Cke.) Wint. Most of them have been based on field surveys in the eastern and east central United States (Chadwick, L. C., 1965; Nichols, L. P., 1963; Nichols, L. P., 1965; and Nichols, L. P., 1967) or on the effects of greenhouse inoculations (Shay, J. R., 1961; and Shay, J. R. and L. F. Hough, 1952). This present survey was undertaken to observe the effects of apple scab under field conditions occurring in northeastern Illinois in 1966, 1967, and 1968.

A survey of crabapples growing at the Morton Arboretum, Lisle, Illinois was made in August of each of the three years to observe the occurrence and severity of apple scab. About 250 species, varieties, and cultivars were examined, most of which were represented by at least three specimens.

The results of this survey are listed in Table 1. The degree of injury was rated from 0 to 3, with 0 indicating no lesions or spots on the leaves and the plant apparently free from apple scab; 1, lesions or spots present on less than one-half of the foliage; 2, lesions or spots present on more than one-half of the foliage with slight defoliation; 3, lesions or spots on most of the foliage accompanied by severe defoliation.

Whenever cedar-apple rust, *Gymnosporangium juniperi-virginianae* Scw., powdery mildew, *Podosphaera* sp., or fire flight *Erwinia amylovora* (Burr.) Winslow, *et al,* were observed, they were noted in the survey as "other diseases." No attempt was made to rate the severity of these infections.

Observations of other authors have also been incorporated in Table 1. The differences in the data reported here, from what other workers have observed, are possibly due to different strains of the causal organism (Bagga, H. S. and D. M. Boone, 1968), variation in climatic conditions, or incorrect identification of plant material.

KIND[a]	Scab 66	Scab 67	Scab 68	Other Disease Observed[b]	Other Authors[c]
Abundance*	..	0	0		Ra
x *adstringens*	2	1	2		Sa, d
ALEXIS	1	1	1		Sa
ALMEY	3	1	2		Sb, d, e
AMISK	1	2	1		Ra, Se
AMUR	1	0	2		
angustifolia	0	0	0	CA	
Anoka*	..	1	..		
ARCTIC DAWN	0	0	0		Ra
x *arnoldiana*	2	1	2		Re, Sb, d
ARROW	3	1	1		Sa, d
x *astracanica*	1	0	0		Ra
x *atrosanguinea*	0	0	1		Rd, e, Sc
Babine*	1	1	1		
baccata	1	0	2		Sc, d
b. aurantiaca*	1	1	1		
b. cerasifera*	..	..	2		
b. cerasiformis*	0	0	1		
b. columnaris	1	1	1		Rd, Sc, e
b. fructo-flava*	1	1	1		
b. gracilis	0	0	0		Sc
b. himalaica	0	0	0		Ra
b. jackii	0	0	0		Rc, d, e
b. lasiostyla*	0	0	..		
b. lutea*	..	0	0		
b. oblonga*	1	0	0		
b. odorata*	2	3	2		
b. 'scab immune'*	0	0	0		
b. 'source'*	1	0	1		
b. stricta*	1	1	..		
b. 'Unusual'*	..	..	1		
BASKATONG	1	1	1		Ra
BEAUTY	1	1	2		Rc
BEDFORD	0	0	1	PM	
Beverly*	0	1	0		
BOB WHITE	0	0	1		Rc, d, e
bracteata	2	1	1		
brevipes	1	1	1		Sc, d
BRIER	1	2	2		
CASHMERE	..	0	1		Sa
CATHAY	..	0	0		Ra
CHEAL'S CRIMSON	1	0	1		Ra, Sd
CHILKO	1	1	1		Ra
COLUMBIA	1	1	1		Sa
coronaria	1	1	1	CA	Sa, d
c. charlottae	..	..	2	CA	Ra, Sd, e
c. dasycalyx	1	1	1	CA	Ra
c. nieuwlandiana	1	1	2	CA	Vd, Sc
c. spinosa*	2	1	1	CA	
COWICHAN	2	1	1		Sa, d
CRIMSON BRILLIANT	2	2	3		Vd, Sb, e
DARTMOUTH	2	1	2		Ra
David*	0	0	1		Rc
x *dawsoniana*	0	1	1	PM	Sb
DELITE	1	0	..		

KIND[a]	Scab			Other Disease Observed[b]	Other Authors[c]
	66	67	68		
denticulata*	1	1	1		Sa
Des Moines*	1	0	1		
DOLGO	0	0	1		Sc, e
DOROTHEA	1	1	2		Rd, e, Sc
E. H. WILSON	2	2	2		Ra
ELISE RATHKE	1	1	2		Ra, d
ELK RIVER	1	1	1	CA	Ra
ELLWANGERIANA	..	0	0		Ra
ERIE	3	0	1		Sa
EVELYN	1	1	1	FB	Rc, d, e
EXZELLENZ THIEL	..	1	1		Sa
FERRILL'S CRIMSON	2	2	3		Rb
FLAME	1	0	1		Sc, d
flexilis*	0	0	0		Ra
floribunda	1	0	1		Rd, e, Sc
FRAU LUISE DITTMANN	0	0	1		Ra
fusca	0	1	1		
Giant Wild Crab*	1	2	3	CA	
GIBB	1	1	0		Ra
glabrata	2	1	1		Ra
glaucescens	1	2	2		Rd, Sa
x *gloriosa*	1	0	1		Ra, d
Golden Gem*	0	0	0		
GOLDEN HORNET	0	0	0	PM	Rb
GOLDFINCH	2	2	1		Rb
GORGEOUS	1	0	0		Rc
GWENDOLYN	1	1	0		Rb
halliana	..	..	2		Ra, d, e
halliana spontanea	0	0	0		Sa
x *hartwigii*	..	1	1		Rb, d
HENRIETTA CROSBY	2	1	2		Sb
HENRY F. DuPONT	1	1	2		Sc, d, e
HILLIER CRAB	0	0	1		Ra, d
HOPA	3	2	2		Sb, d, e
hupehensis	0	1	1		Rc, d, e
hybrid 'scab immune'*	1	0	0		
hybrida*	1	0	1		
ioensis	1	1	1	CA	Sa
i. 'Clinton'*	2	2	2	CA	
i. fimbriata	0	0	0	CA	Ra
i. klemii	1	1	2	CA	Ra, Vd, Se
i. plena	1	1	2	CA	Sb, d
i. plena Dwarf*	2	1	2	CA	
i. Rocky Glen*	..	1	1	CA	
IRENE	3	3	3		Sa
JAY DARLING	2	2	3		Vd, Sb
JOAN	2	2	2		Sa
Jubilee*	1	2	1		Ra
kansuensis	1	0	0		Ra
(kansuensis x toringoides)*	..	..	2		
KATHERINE	1	1	1		Rd, Sb, e
Kibele*	1	1	1		
KINGSMERE	1	1	1		Ra
kirghisorum*	1	1	2	PM	Ra
KIT TRIO	3	2	..		Ra

KIND[a]	Scab 66	Scab 67	Scab 68	Other Disease Observed[b]	Other Authors[c]
Kohankie*	1	1	0		
KOLA	1	1	1	CA	
lancifolia	1	1	1		Vd, Sa
Linda*	1	1	1		
Lings*	1	1	2	CA	
LISA	..	1	1		
LISET	0	0	1		Rb, d, e
x *magdeburgensis*	1	1	0		Ra
MAKAMIK	0	0	0		Rc, d
malifolia*	1	1	0		
MARSHALL OYAMA	1	0	1		Rd, Sc, e
MARTHA	2	1	2		Ra
MARY POTTER	0	0	0		Rc
MATHEWS	1	1	2	CA	Ra
x *micromalus*	0	0	0		Ra, d, e
MONTREAL BEAUTY	2	1	2		Sa
Morden (19-85)*	0	0	1		
MORTON	1	1	1	CA	Ra
MRS. BAYARD THAYER	2	1	2		Ra
NANCY TOWNSEND	..	1	1		Ra
NEVILLE COPEMAN	2	1	1		
NEVIS	1	1	2		Ra
Nicoline*	..	..	1		
Northland (Minn. 1423)*	1	0	0		
NOVA	1	1	1	CA	Rb
OAKES	1	3	3		Ra, Sd
OEKONOMIERAT ECHTERMEYER	1	2	3		Sb, d
Okanagan*	1	1	1		
OPORTO	0	0	0		Ra
ORANGE	2	1	1		Ra
Orchid*	0	0	0		
ORMISTON ROY	0	0	1		Rc
orthocarpa*	..	0	0		
OSMAN	..	1	0		Ra
PATRICIA	1	1	1		Sb
PEACHBLOW	0	0	0		Ra
Pink Beauty*	0	0	0		Sd, e
PINK EYE	1	1	1		Ra
PIOTOSH	1	1	1		Ra
PIXIE	2	2	2		Rb
platycarpa	1	1	0	FB	Sa
p. hoopesii	..	1	2		Ra
Ponass*	1	1	2		
Prairie Rose*	1	1	2	CA	Ra, e
prattii	0	0	0		
Pretty Marjorie*	2	1	1		Ra
PRINCE GEORGES	0	0	0	CA	Rd, Sb, e
PROFESSOR SPRENGER	0	0	1		Rb
PROFUSION	1	0	1		Ra
prunifolia	0	0	0		Rb
p. fastigiata	1	1	1		Ra
p. fructu-coccinea*	1	1	2		Ra
p. rinkii	0	0	2	PM	Sb, d
p. xanthocarpa*	1	1	0		Ra
pumila	1	1	1		

KIND[a]	Scab 66	Scab 67	Scab 68	Other Disease Observed[b]	Other Authors[c]
p. apetala	1	1	1		Ra
p. niedzwetzkyana	1	1	1		Ra, Vd
p. paradisiaca aureus*	1	1	2	FB	
p. p. ruberrima*	1	1	2		
p. pendula	1	1	2		
p. translucens	2	1	..		Ra
PURPLE WAVE	1	0	2	FB	Ra, e, Vd
x *purpurea*	0	0	1		Ra, d
x *p. aldenhamensis*	2	2	1		Sb, d, e
x *p. eleyi*	3	2	3		Sb, d, e
x *p. kornicensis*	0	0	0		Ra
x *p. lemoinei*	1	1	1		Rd, e, Sc
QUEEN CHOICE	2	1	2		Ra
Radiant*	3	2	3		Sc
REDFLESH	1	2	2		Ra
REDFORD	1	1	1		Ra
RED JADE	1	0	1		Rd, Sc, e
Redman*	0	1	0		
RED SILVER	1	1	2		Rd, Sb
RED SPLENDOR	0	0	0		Rc
RED TIP	2	1	..	CA	Ra
x *robusta*	..	1	0		Rd, Sc
x *robusta erecta*	0	0	0		Sb, d
x *r. persicifolia*	1	0	0		Rc, d
Rockii	0	0	0		Ra
RONDO	2	2	3	CA	Ra
ROSSEAU	1	1	1		Rc, d
RUDOLPH	1	0	1		Ra, Vd
sargentii	0	0	1		Rd, e, Sc
sargentii (Ill.)*	0	0	1		
s. rosea	0	0	0	FB	Rb, d, Se
x *scheideckeri*	1	1	0		Re, Vd, Sc
SCUGOG	0	0	1		Ra, Sd
SEAFOAM	..	..	2		Rc
sieboldii	1	1	2		Ra
s. arborescens	1	1	1	PM	Rc
sikkimensis	1	1	1		Ra, d
SIMCOE	0	0	0		Ra, Sd
SISSIPUK	1	1	1		Rd, Sc
SNOWBANK	0	0	0		Rc
x *soulardii*	1	0	1		Sa, d
spectabilis	1	1	0		Sa
s. albi-plena	1	1	0		Sc, d, e
s. riversii	..	0	0		Vd, Sc, e
STRATHMORE	2	2	2		Sb, d, e
STRIPED BEAUTY	1	0	1		Ra
x *sublobata*	1	1	2		Sa, d
sylvestris	1	1	1		Ra
s. plena	1	1	0		
TANNER'S VARIETY	2	1	1		
THOMAS ROLAND	1	1	2	FB	Sa
TIMISKAMING	1	0	..		Ra
TOMIKO	2	1	2		
toringoides	1	1	1		Rc
t. macrocarpa	1	1	2		Sb

KIND[a]	Scab 66	Scab 67	Scab 68	Other Disease Observed[b]	Other Authors[c]
TOSHPRINCE	1	1	0		Ra
TRAIL	0	1	1		
transitoria	1	..	2		Rd
VAN ESELTINE	1	1	1		Rd, Sc, e
Van Houttei*	0	0	1		
VEITCH'S SCARLET	1	1	1		Ra
VIRGINIA SEEDLESS	..	1	1		Sa
WABISKAW	1	1	2		Ra, Vd
WIERDAK	..	..	1		
Wild Red Crab*	..	1	1		
WILLIAM ANDERSON	0	1	1		Ra
WILLIAM SIM	..	1	1	FB	Sb
WINTERGOLD	0	0	0		Rc
WISLEY CRAB	1	1	1		Ra
WYNEMA	2	2	3		Ra
yunnanensis veitchii	1	1	1		Ra, d
ZITA	..	1	..		
zumi	1	1	1		Ra, d
z. calocarpa	0	0	0		Rc, d, e
spec 19039*	1	..	1		
spec AA 20*	1	1	1		
spec AA 33340*	1	1	1		
spec D1*	1	0	1		
spec D2*	0	0	0		
spec D4*	..	..	0		
spec L. B. 1*	1	0	0		
spec VC 4*	2	1	2		Ra
spec W 12*	0	0	0		

TABLE 1. — Susceptibility and degree of injury to crabapples from apple scab.

(a) The nomenclature of the species, which are italicized, is from Rehder (1940, 1949), that of the cultivars, which are in capitals, is from Krussmann (1962) and Wyman (1955). Names followed by an asterisk (*) represent taxa that are new, obsolete, or discarded.

(b) Occurrence of other diseases:
CA — Cedar-apple rust
FB — Fire blight
PM — Powdery mildew

(c) Reported by others as R — resistant, V — variable, or S — susceptible to apple scab.
a Nichols, 1963
b Nichols, 1965
c Nichols, 1967
d Chadwick, 1965
e Shay, 1961

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THE INFLUENCE OF COLD AND CHEMICAL TREATMENTS ON THE AFTERRIPENING OF APPLE SEEDS

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ABSTRACT. — Seeds of nine apple cultivars and three crabapple species were soaked in three concentrations each of elgetol, ethylene chlorohydrin, gibberellic acid, and thiourea, in addition to a distilled water check. After soaking, the seeds were stratified for 12, 8, 4 or 0 weeks at 4 C. For all cultivars and species, the 8- and 12-week cold treatments were effective in breaking the rest period of apple seeds. The 4-week cold treatment was also effective in the *Malus sargenti*, *M. toringoides* and Jonathan experiments. In the absence of cold treatment, seed germination was negligible. The chemical treatments factor was highly significant by the F-test with 11 cultivars and species. However, no chemical treatment gave greater germination than distilled water while several elgetol and ethylene chlorohydrin treatments, depending upon the cultivar, markedly inhibited germination. Apparently, any positive chemical effect on afterripening was due to the influence of water in the aqueous solutions.

The term "dormancy" in seeds is used to describe two inactive conditions. One dormant condition results from an unfavorable environment for growth, and imposed blocks within the seed induces a rest period causing the second type of dormant condition. According to Pollock and Toole (1961), germination blocks in apple seeds are primarily of the latter type of dormancy.

Pollock and Toole (1961) and Kawase (1958) reported that apple seeds germinated only after undergoing an 8- to 12-week period of afterripening or stratification while moist at a temperature near 40 F. However, Cockran, Cooper and Blodgett (1961) reported that apple seeds required only six weeks or less for afterripening.

Luckwell (1952) found that soaking apple seeds and embryos in several thiourea and ethylene chlorohydrin solutions was ineffective in breaking the rest period of apple seeds.

Donoho and Walker (1957) and Chao and Walker (1966) reported that gibberellic acid eliminated the chilling requirements of peach seeds. Tukey and Carlson (1945) found that thiourea broke the dormancy of Lovell peach seeds.

Bukovac (1962) described abnormal apple seedling growth when the seedlings were treated with potassium gibberellate. Chao and Walker (1966) stated seedling growth was often stunted, with short internodes and abnormal leaves, when peach seeds were soaked in potassium gibberellate. Tukey and Carlson (1945) found that peach seedlings were dwarfed, with shortened internodes and malformed leaves, following seed treatment with thiourea.

Dermen (1936) and Tukey and Brase (1938) reported poor germination of *Malus hupehensis* seeds,

which they attributed to a triploid condition and apomictic seed development.

The purpose of this investigation was to determine the effect of cold and/or chemical treatments upon the afterripening period of apple seeds.

Materials and Methods

Twelve experiments were conducted simultaneously with open pollinated seeds of the following cultivars and selections of the apple (*Malus pumila* Mill.): Cortland, Crandall, Gallia, Golden Delicious, Jonathan, Red Flesh Crab, Solid Red Winesap, Starkrimson, York-A-Red, *M. hupehensis, M. sargenti* and *M. toringoides.* Fruits of the nine apple cultivars and three crabapple species were collected. The seeds were removed from the fruit and stored dry at room temperature.

Twenty-seed lots of each apple cultivar and crabapple species were soaked for 24 hours in each of 13 chemical treatments (Table 1)—the concentrations were selected in geometric progression. After soaking, the solutions were decanted, and the seeds were stratified at 4 C in closed plastic bags of moist sawdust. The soakings were performed at 4-week intervals—so timed for simultaneous emergence from the afterripening treatment.

Table 1. — Chemical treatments in aqueous solutions.

Chemical	Concentrations %	%	%
Elgetol	0.25	1.00	4.00
Ethylene Chlorohydrin	0.25	1.00	4.00
Thiourea	0.25	1.00	4.00
Gibberellic Acid	0.02	0.10	0.50
Distilled Water (Check)	100.00		

Within five days of the last soaking, the 12 experiments were planted in a sterilized sand bed in a sash house for seed germination at approximately 20 C. A split-plot design with two replications and 10 seeds per plot was used for each experiment. Cold treatments were assigned to the main plots and chemical treatments were assigned to the subplots.

Two, three, five and six weeks after planting, the seedlings were pricked-out of the sand bed and germination numbers were recorded. Observations were made on the growth of the seedlings for the next four months.

Results and Discussion

The cold treatments factor, the chemical treatments factor and the cold/chemical treatment interactions factor were significant at the 1% level by the F-test for all experiments, as exemplified by the Jonathan analysis (Table 2), except the

Table 2. — Analysis of variance of Jonathan seed germination.

Source	df	MS	F
Main Plots	7		
Replications	1	0.1	0.1 ns
Cold Treatments	3	312.6	312.6 **
Error (a)	3	1.0	
Subplots	103		
Chemical Treatments	12	26.8	16.8 **
Cold x Chem. Treatments	36	9.0	5.6 **
Error (b)	48	1.6	

TABLE 3. — Analysis of variance of *Malus hupehensis* seed germination.

Source	df	MS	df
Main Plots	7		
Replications	1	0.4	0.3 ns
Cold Treatments	3	29.2	18.3 **
Error (a)	3	1.6	
Subplots	103		
Chemical Treatments	12	5.7	1.9 ns
Cold x Chem. Treatments	36	3.0	1.0 ns
Error (b)	48		

M. hupehensis experiment. With the *M. hupehensis* experiment (Table 3), only the cold treatments factor was significant at the 5% level by the F-test. The lack of significance in the *M. hupehensis* species was attributed to the overall poor germination of *M. hupehensis* which agreed with the reports by Dermen (1936) and Tukey and Brase (1938).

The 4-week cold treatment was active in afterripening in the Jonathan experiment (Table 4), but germination increased with additional cold treatment. The influence of cold treatments on seed germination of *M. sargenti* and *M. toringoides* was similar to the influence of cold treatments on Jonathan seed germination. However, no difference was observed between the 8- and 12-week cold treatments in the two crabapple experiments.

TABLE 4. — Comparisons of the influence of cold treatments on Jonathan seed germination.

Cold Treatment Weeks	Mean[1,2]
12	7.2 a
8	5.8 b
4	1.2 c
0	0.0 d

[1] Mean number of seeds germinated from 10-seed plot.
[2] Treatments followed by common letters are not significant at the 5% level by Duncan's Multiple Range Test.

TABLE 5. — Comparisons of the influence of cold treatments on Starkrimson seed germination.

Cold Treatment Weeks	Mean[1,2]
12	7.5 a
8	6.3 b
4	0.7 c
0	0.3 c

[1] Mean number of seeds germinated from a 10-seed plot.
[2] Treatments followed by common letters are not significant at the 5% level by Duncan's Multiple Range Test.

The seeds of 'Starkrimson' (Table 5) did not germinate until they received eight weeks of cold treatment and germination increased with additional cold treatment. The germination response of Cortland, Crandall, Gallia, Golden Delicious, *M. hupehensis*, Red Flesh Crab, Solid Red Winesap and York-A-Red seeds to cold treatment was similar to the germination response of Starkrimson seeds. The 8-week cold treatment permitted germination equal to the 12-week cold treatment in the latter four experiments.

Evidently, the amount of cold treatment necessary to break the rest period differs with the cultivar and presumably accounts for the discrepancies in the findings of Pollock and Toole (1961), Kawase (1958) and Cockran, Cooper and Blodgett (1961).

No chemical treatment induced greater germination than distilled water as exemplified by the Jonathan and Starkrimson experiments

TABLE 6. — Comparisons of the influence of chemical treatments on Jonathan seed germination.

Chemical Treatment % aqueous		Mean[1,2]
0.50	Gibberellic Acid	6.0 a
Ck	Distilled Water	5.5 ab
0.10	Gibberellic Acid	5.4 ab
0.02	Gibberellic Acid	5.3 a-c
0.25	Thiourea	5.0 a-c
4.00	Thiourea	4.0 bc
1.00	Thiourea	3.6 b-d
0.25	Elgetol	3.4 cd
4.00	Ethylene Chlorohydrin	2.1 de
4.00	Elgetol	2.0 de
0.25	Ethylene Chlorohydrin	1.9 de
1.00	Elgetol	1.3 e
1.00	Ethylene Chlorohydrin	0.5 e

[1] Mean number of seeds germinated from a 10-seed plot.
[2] Treatments followed by common letters are not significant at the 5% level by Duncan's Multiple Range Test.

TABLE 7. — Comparisons of the influence of chemical treatments on Starkrimson seed germination.

Chemical Treatment % aqueous		Mean[1,2]
0.02	Gibberellic Acid	5.5 a
0.10	Gibberellic Acid	5.1 a
0.25	Thiourea	5.0 a
1.00	Elgetol	5.0 a
Ck	Distilled Water	4.8 ab
0.50	Gibberellic Acid	4.3 a-c
4.00	Thiourea	4.1 a-c
0.25	Elgetol	3.9 a-c
4.00	Ethylene Chlorohydrin	3.8 a-c
1.00	Thiourea	3.1 a-d
4.00	Elgetol	1.9 b-d
0.25	Ethylene Chlorohydrin	1.3 cd
1.00	Ethylene Chlorohydrin	0.6 d

[1] Mean number of seeds germinated from 10-seed plot.
[2] Treatments followed by common letters are not significant at the 5% level by Duncan's Multiple Range Test.

(Tables 6 and 7). Generally, all concentrations of gibberellic acid and thiourea gave germination equal to the check, while several concentrations of elgetol and ethylene chlorohydrin, depending upon the cultivar, markedly inhibited germination.

The results of the chemical treatments agreed with those of Luckwell (1952). Apple seed rest period was not eliminated by chemical treatments which broke the rest period of peach seeds (Donoho and Walker, 1957; Chao and Walker, 1966; Tukey and Carlson, 1945).

The cold/chemical treatment interactions, although highly significant, were of little practical importance. Generally, the three gibberellic acid concentrations, the three thiourea concentrations and the check gave equal germination within the cold treatments, whereas the relative germination response from a specific elgetol or ethylene chlorohydrin concentration varied inconsistently with the cold treatments. Commonly, no chemical treatment gave greater germination than the distilled water check within a cold treatment.

In these experiments, growth was apparently normal and no growth differences were observed between seedlings resulting from any combination of cold and/or chemical treatments. These results did not agree with those of Bukovac (1962), Chao and Walker (1966), and Tukey and Carlson (1945).

ACKNOWLEDGMENTS

This study represents a portion of a thesis submitted in partial fulfillment of the requirements for the Master of Science degree for the senior author at Southern Illinois University. This research was supported by the Illinois Horticultural Experiment Station, Carbondale, Illinois. This paper is Publication No. 30, published with the approval of the Horticulture Department, University of Illinois, and the Plant In-

dustries Department, Southern Illinois University.

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SUCCESSION AND SPECIES DISTRIBUTION IN RELATION TO EROSION IN SOUTHERN ILLINOIS

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ABSTRACT. — Fields abandoned for 30-35 years are common in southern Illinois. Abandonment is related both to the inherently poor quality of the soil and to erosion. This study compares the vegetation of two fields abandoned for the same time yet different in the degree of erosion. In the severely eroded field, large gullies were present, the tree layer was poorly developed and individual trees and shrubs were generally small; the herbaceous cover was high and the number of species was high; the reverse was true for the moderately eroded field. Severe erosion has led to the formation of many microhabitats in which different plant species are present. These species were distributed according to the degree of erosion, and some of them appear to be good indicators of erosion.

Large areas of abandoned agricultural land occur in the eastern part of the Shawnee Hills of southern Illinois. Most of this area was covered by oak-hickory forests before settlement by European immigrants. The loess soils, on Mississippian bedrock, are usually shallow, highly weathered and leached, rather acid, and low in nutrients. The upper parts of the profile are well drained, and water moves readily downward until it reaches the firm fragipan which is typical of these soils (Fehrenbacher and Odell, 1959).

The major soil type in the area is Grantsburg silt loam (301), a light colored soil developed from 80 in. of loess over sandstone residum or sandstone bedrock. Much of this area is under forest vegetation with 2-18 percent slopes (Fehrenbacher and Walker, 1964). This soil is highly weathered and leached, has bisequal profile and the most developed fragipan in the area (Grossman, Fehrenbacher, and Beavers, 1959). Its noneroded phase has an A_p layer (0-6 in.) of dark brown friable silt loam; an A_2 (6-12 in.) of yellowish brown friable silt loam; a B_1 (12-16 in.) of firm heavy silt loam; a B_2 (16-23 in.) of yellowish brown silty clay loam. Below this is a A'_2 (gray layer) which is about 4 in. thick and consisting of firm silty clay loam with light gray coatings; the B'_2 (27-34 in.) is mottled very firm silty clay loam with coarse prismatic structure. Below this is the B'_3 "fragipan", A layer of high bulk density, low clay content and massiveness. The layer is very hard and brittle when dry and greatly restricts water movement and root penetration. Erosion is extensive in the area because of the restriction of water movement into the soil, the relatively high rainfall (120 cm) and the rugged topography. As a result, the effective part of the soil profile is very shallow and the plants are frequently subjected to drought.

Erosion and poor soils have led to reduction in crop yields and to abandonment of fields. When compared with other areas of similar vegetational trends, secondary suces-

sion is much slower on these fields (Bazzaz, 1968). Different degrees of erosion have created many microhabitats in which different plant species predominate. This study was conducted to determine if correlations between degree of erosion, plant cover and succession, and the distributions of various plant species could be made. Also of interest was possible use of these species as indicators of severity of erosion.

Methods

Two fields located in Pope County and abandoned for thirty years were studied. Both were located on Grantsburg silt loam soil type. One field was located on a 4-7% slope and was moderately eroded, with some of the A horizon remaining, whereas the other field was located on a 7-12% slope and was severely eroded. The soil type of the first is therefore designated as (301 C) and that of the second as (301 D) (Fehrenbacher and Walker, 1964). The sampling plots were laid out as follows: for trees, 10-by 10 m; for shrubs, 4-by 4 m; and for herbs, 2-by 1 m. Twenty-five plots of each size located on both sides of an east-west compass base line were used in each field. Species and dbh values for trees (above 2.5 cm) were recorded; species and cover values for herbs and shrubs were estimated. Basal area and density were calculated for trees; density and cover were obtained for shrubs; and cover was estimated for herbs (Table 1). Nomenclature is that of Jones (1963).

Examination of several gullies in the area suggested a possible correlation between the distribution of some plant species and degree of erosion. To test this possibility the vegetation of a part of a large but shallow gully was mapped in a transect 4.5 m long and 0.5 m wide, extending from the bottom to the stable, upland herbaceous community dominated by *Andropogon virginicus* and *Solidago nemoralis*. The transect was divided into 10-by 10 cm units and each mapped on coordinate paper, indicating species, numbers, and positions of individuals, and their relative cover. Frequency and density values were calculated for species present. The degree of erosion along the transect was estimated, using as a reference the "gray layer" of the soil.

Correlation coefficients (DeVries, 1954) were calculated for the three important species in the gully using the formula:

$$r = \text{Sin } (T\text{-}90^\circ) \text{ and}$$

$$T = \frac{A\,D - 3\,C}{P+Q+R+S} \text{ where}$$

A = No. of times species X and Y occur together; D = No. of times neither is present; C = No. of times Y occurs without X; P = No. of times Y is found in sampling; Q = Total No. of samples minus R; R = No. of times X occurs without Y; S = Total No. of samples minus P.

Results and Discussion

The tree layer was dominated in both fields by persimmon (*Diospyros virginiana*), sassafras (*Sassafras albidum*), red cedar (*Juniperus virginiana*), and winged elm (*Ulmus alata*). *Crataegus mollis, Carya tomentosa, Ulmus rubra, Nyssa sylvatica,* and *Gleditsia triacanthos*

were also present. These were found mainly on the stable, less eroded parts of the fields. The first two dominant species, which form clonal populations by root proliferation, were mostly in clumps. The same woody species plus *Rhus copallina* were found in the shrub layer. The herbaceous layer consisted mainly of *Andropogon virginicus, Smilax glauca, Aristida dichotoma, Potentilla simplex, Rubus occidentalis, Panicum dichotomum, Solidago nemoralis, Andropogon elliottii* and *Solidago altissima.*

Erosion greatly affects plant growth. In addition to loss of soil profile and change in pore space relationships erosion increases runoff and organic matter losses from the soil (Bazzaz, 1963) and lowers the infiltration rates. The degradation in soil characteristics leads to slower establishment and growth of plants. Comparisons of the basal area, the density values, and the plant cover in the two fields (Table 1) clearly show that the moderately eroded field has a better developed tree layer. Slow growth of plants on the severely eroded field is also indicated by the low stature of individual trees. For instance, although persimmon had a higher density on the severely eroded field (224 vs 144/hectare), its basal area was lower (0.047 vs 0.058 m^2) reflecting smaller individuals. The herb layer was better developed in the severely eroded fields, as is indicated by its higher total cover (Table 1).

The number of species (Table 1) in the tree and the shrub layers was higher in the moderately eroded field, indicating that only erosion tolerant species are capable of becoming established in the more severe habitats. In the herb layer, however, there was a larger number of species in the severely eroded field. This was correlated not only with the lower tree and shrub cover of the field, but was also related to the diversity of microhabitats created by erosion.

Aristida, which dominated the gullies (Fig. 1, Table 2), was more important in the severely eroded field, having a cover value of 8.5 percent/m^2 and a frequency of 56%. In the other field its cover and frequency were 3.9 percent/m^2 and 44%, respectively. In the severely eroded field there was also a higher lichen cover (2.1 vs 0.05 percent/m^2) and more rosettes of *Solidago nemoralis*

TABLE 1. — Vegetation of two abandoned fields with different degrees of erosion.

		Moderately Eroded	Severely Eroded
Tree layer	Total Density/hectare	1648	964
	Total Basal Area (m^2)	0.511	0.321
	No. of species	12	7
Shrub layer	Total Density/hectare	6250	2100
	Total percent cover/m^2	0.712	0.700
	No. of species	7	8
Herb layer	Total percent cover/m^2	92.5	118.3
	No. of species	26	34

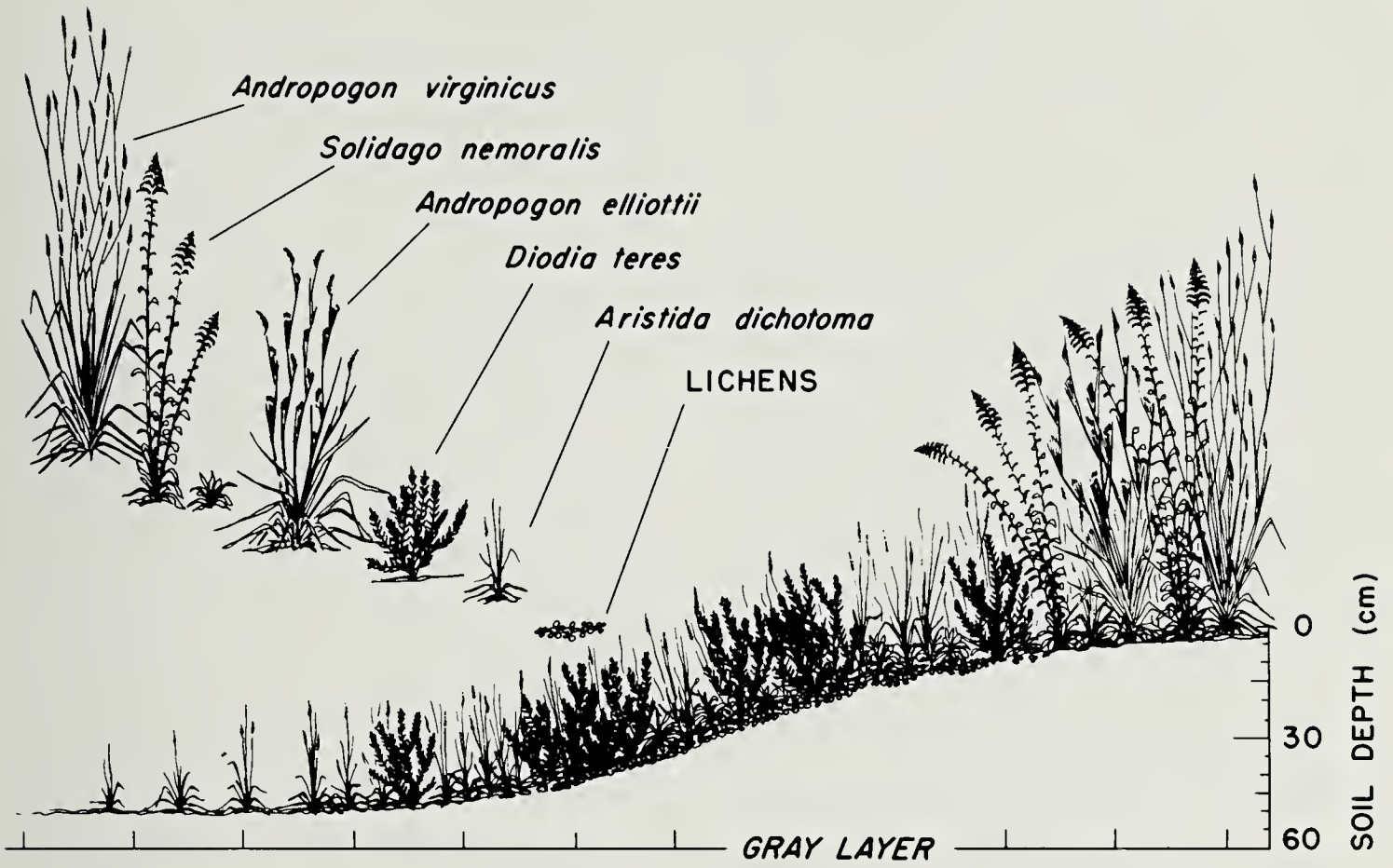

FIGURE 1. Diagramatic representation of species distribution in a gully.

TABLE 2. — Frequencies and densities of species found in a gully.

Species	Frequency %	Density/m²
Aristida dichotoma	88.0	645.0
Lichens	40.3	40.0
Diodia teres	31.8	83.0
Crotalaria sagittalis	7.3	16.0
Crotonopsis elliptica	7.1	14.0
Strophostyles umbellata	6.4	12.0
Solidago nemoralis (rosettes)	6.4	9.0
Andropogon elliottii	6.2	2.0
Panicum sp.	1.8	8.0
Smilax glauca	1.2	4.0

that failed to develop flowering stems. Both the lichens and *Solidago* rosettes are found in gullies (Fig. 1).

Table 2 and Fig. 1 show that in the gullies, *Aristida* was the dominant species; it was followed by lichens and *Diodia teres*. At the bottom, where the profile was severely eroded to a depth of 50 cm, *Aristida* was the dominant species, although individuals were scattered (Fig. 1). A few individuals of *Digitaria sanguinalis, Strophostyles umbellata,* and *Crotalaria sagittalis* were found with *Aristida* in the bottom area. Near the side of the gully, where there was less water action, a few lichens and scattered individuals of *Diodia* and *Euphorbia corollata* occurred with *Aristida*. Lichens were more important farther up, and

other species including *Crotonopsis elliptica* and *Cassia fasciculata* occurred. There was also an increase in the importance of *Diodia.*

On the side of the gully, a relatively steep slope, lichens (mainly species of *Cladonia*) were very important and covered almost all the soil surface. The number of plant species also increased but not as much as the number of individuals, especially of *Diodia* and *Aristida.* On the upper half of the gully slope *Smilax glauca* and rosettes of *Solidago nemoralis* occurred. The *Smilax* was very poorly developed and the rosettes of *Solidago* were small. This apparently indicates the severity of microenvironment of these areas with very low nutrient levels and little available water. At the top of the gully slope, lichens and *Aristida* were less important. However, there was an increase in *Crotonopsis, Smilax,* and rosettes of *Solidago.* Where the topsoil was completely removed and the B horizon was at the surface, small clumps of *Andropogon elliottii* appeared. In less disturbed sites *A. elliottii* was better developed, lichens decreased greatly and a few individuals of *Panicum* sp. occurred. A stable community association of *Andropogon virginicus* and *Solidago nemoralis* was found on sites where there was some topsoil left. Here there was reduction both in the number of species and the number of individuals present.

It is evident from this study that different species were distributed according to the degree of erosion and the stability of the site. *Aristida dichotoma* was found throughout, and was almost the only plant to tolerate extreme instability caused by erosion. Lichens were excluded from the bottom of the gully, probably because they were not attached firmly to the soil and thus washed away easily.

Andropogon virginicus, the dominant of the stable community, was completely absent where no topsoil was left and was a site indicator in the abandoned fields. In southern Illinois, as well as in other areas (Allard, 1942), this species was found only in fields with some topsoil.

Andropogon elliottii indicates more severe erosion. It was found on sites where *A. virginicus* did not grow. *Diodia teres* was quite important in the lichen zone, and only few individuals occurred in the stable community.

Although *Aristida dichotoma* was distributed throughout, it was more prevalent in the area where the lichen cover was thick (Fig. 1). This was indicated by its relatively high correlation coefficient with lichens (Table 3). *Diodia,* however, was more associated with lichens ($r = 70\%$). That *Aristida* was found in larger numbers in the *Diodia* area because of the association of both *Aristida* and *Diodia* with lichens, and not because of direct association of the two, was indicated by the low correlation coefficient between *Aristida* and *Diodia* ($r = 21\%$). Except where the water action is very frequent lichens apparently stabilize the soil surface, reduce erosion, and function as a seed bed where the seeds of *Diodia* and *Aristida* germinate. When lichen mats were brought to the greenhouse, put on sterile soil, and watered adequately,

TABLE 3. — Correlation coefficients of the important species on eroded sites.

	Lichen	*Diodia*
Diodia	70	
Aristida	43	21

large numbers of *Aristida* and *Diodia* plants developed.

Apparently erosion is a very important factor affecting succession in southern Illinois. It is also evident that the degree of erosion controls the distribution of some plant species in gullies. However, a complete understanding of this distribution must take into account the tolerances of these plants to their different microenvironments, including their possible biochemical interactions. Erosion could be reduced successfully by planting pines in these fields since pine litter protects the soil surface quite well (Bazzaz, 1969).

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NOTES AND NEWS

DR. CLARENCE SMITH HONORED

JOAN HUNTER
Illinois State Museum, Springfield, Ill.

Dr. Clarence R. Smith, physics professor of Aurora College, Aurora, was honored at commencement exercises on May 17, 1969, by having the lecture hall in the Stephens Hall of Science named in his honor. Professor Smith, as he is respectfully known by former students and townspeople alike, is a student and teacher of great versatility. From the many facets of his curiosity have emerged a collection of more than 300 species of beetles, photographs of eclipses, collections of data relating to meteorites, recognition as an authority on the mastodon in Illinois, knowledge of trumpeter swans and optics. In addition, he has collections of old books, reptiles, mammals, and amphibian fossils of the Aurora area.

Dr. Smith is in charge of the weather station near Stephens Hall and supplies daily information to the Chicago Weather Bureau. He is on the board of the Aurora Historical Society of which he was a founder, is a member and past officer of the Fox Valley Astronomical Society, and is a life member of the Fox Valley Rocks and Minerals Society. He has been listed in the American Men of Science since 1927 and in Who's Who in the Midwest since 1952.

Dr. Smith graduated from Aurora College (then Mendota College) in 1914; he received his M.S. degree from the State University of Iowa in 1923; and in 1960, the year of his official retirement (he still teaches four hours per week), he received an honorary D. Sc. degree from Aurora College.

He has been a member of the Illinois State Academy of Science since 1921 and has presented papers ranging from "Mastodon and Other Finds at Aurora" to "A Laboratory Switchboard at Low Cost." He served as chairman of the Physics Section in 1950.

Congratulations from the Academy are due this outstanding teacher who has spent a lifetime in seeking and dispensing knowledge, as well as inculcating the joy of achievement in his students.

SECRETARY'S REPORT FOR THE YEAR — APRIL 27, 1968 - APRIL 25, 1969

COUNCIL MEETINGS

The Council held four meetings during the year, as stipulated in the Constitution.

First Council Meeting. — The first Council meeting was held in the University Center of Southern Illinois University at Edwardsville on Saturday morning April 27, with President William C. Ashby presiding. Fifteen persons were present.

The following persons were appointed to serve as chairmen of special committees: Dr. Charles K. Hunt, Accreditation and Certification; Miss Elnore Stoldt, Junior Academy; Mr. Milton D. Thompson, Nominations; Dr. G. R. Yohe, Permanent Central Office; Miss Joan Hunter, Planning; Dr. Harold M. Kaplan, Resolutions; Dr. Fred Rolf, Visiting Lecturer. The President was directed to appoint the Second Vice President.

A major consideration of the meeting was the possibility of changes in the format of the annual meeting.

A petition from Dr. Weatherbee for the organization of a Collegiate Sub-Section in the Chemistry Section was approved by the Council.

Second Council Meeting. — As part of the modified format the Fall Council Meeting was held at the site of the next annual meeting, Millikin University, on November 9. Twenty-five members were present.

The relationship between the Junior Academy and the Illinois State Academy of Science, in regard to the Permanent Central Office Proposal and subsequent operation, was carefully examined and approved.

At 11:02 a.m. a tremor was felt which caused the meeting room to rock and roll for about 20 seconds.

A motion was passed which directed President Ashby to appoint an ad hoc Committee on Investments.

Third Council Meeting. — The Winter Council Meeting was held at the Illinois State Museum, Springfield on Saturday February 8, 1969. Twenty members were present.

President Ashby reported that an ad hoc Committee on investments had been formed. Constitutional revisions were presented to the Council. The substance of these amendments and revisions to be sent to the membership and voted upon at the Annual Business Meeting.

Fourth Council Meeting. — The Spring Council Meeting was held at the Holiday Inn, Decatur, on Thursday evening following the Council Dinner. Twenty-two members were present.

The Council voted to accept a proposal from the Illinois Legislative Council to cooperate in a feasibility study for the development of liaison with the Illinois State Academy of Science for use of the members' scientific skills for assistance in solving governmental problems. Milton D. Thompson, Director of the Illinois State Museum, was authorized to serve as the Academy's liaison officer.

The budget for 1969 was amended to increase the amount of the Printing Transactions line item to $2700.00 in order to cover the increased costs of publication (Vol. 62, No. 2.).

The Council approved the investment of up to $5000.00 in the T. Rowe Price Mutual Fund.

Dr. Fred Rolf's report on the Visiting Lecturer Program indicated 75 assignments were made. The Council hopes this program will continue to be successful and expand.

THE 62ND ANNUAL MEETING

General Meeting. — The general session of Friday morning April 25, 1969, held in Albert Taylor Hall, was called to order by President Ashby.

Dean J. Roger Miller, Vice President and Dean of the University gave the address of welcome.

Dr. Ashby gave the President's Report which indicated the activities and accomplishments of the Academy in the past year.

Dr. R. Maurice Myers introduced the speaker and distributed question forms for the discussion session. Dr. Richard G. Browne gave the address, "Illinois Higher Education, Past, Present, and Future." A discussion session followed.

On Friday evening in Albert Taylor Hall, Dr. Harvey I. Fisher, Chairman, Department of Zoology, Southern Illinois University, Carbondale gave the Annual Public Lecture, "The Laysan Albatross", which included his award-winning film.

On Saturday morning April 26, Drs. Robert A. Evers, Illinois Natural History Survey, Urbana; Randy Boggess, University of Illinois, Urbana; and Charles Kendeigh, University of Illinois, Urbana; conducted a botany field trip to Allerton Park near Monticello.

Technical Sessions. — On Friday, April 25, the following papers were presented:

Anthropology

Room M-313, Liberal Arts Hall
Martin McGuire, Chairman
Illinois State University, Normal

No report received.

Aquatic Biology

Room 202, Scovill Science Hall
B. J. Mathis, Chairman
Bradley University, Peoria

Primary Productivity in the Middle Illinois River. S. A. Myers and B. J. Mathis, Bradley University, Peoria.

Causal Observations of Protozoa in Tertiary Sewage Treatment. Ralph L. Evans, State Water Survey, Peoria.

Oxidative Phosphorylation of Bluegill Liver Mitochondria in the Presence in some Aquatic Herbicides. Robert C. Hiltibran, Illinois Natural History Survey, Urbana.

Development of Trunk Neuromasts. Robert R. Stephens, Bradley University, Peoria.

Botany

Section A

Room 216, Scovill Science Hall

Robert H. Mohlenbrock, Chairman
Southern Illinois University, Carbondale
Walter E. Schmid, Presiding
Southern Illinois University, Carbondale

Some Morphological Effects of Morphactin on Plantlet Initiation and Development in *Bryophyllum* spp. Patricia Hanzely, Southern Illinois University, Carbondale.

Observations of some Phenotypic Traits in *Datura* Species and Strains. Mark J. Solomon and Frank A. Crane, College of Pharmacy, University of Illinois, Chicago.

Alkaloids in the Germinating Seedling of Poppy. Frank A. Crane, College of Pharmacy, University of Illinois, Chicago, and James W. Fairbairn, University of London.

Effect of Ultraviolet Light on the Growth of *Helminthosporium oryzae*. Stephen H. Buck and Herbert L. Monoson, Bradley University, Peoria.

Effect of Chelating Agents on Ion Transport in Excised Barley Roots. Walter E. Schmid, Southern Illinois University, Carbondale.

Changes in Characteristics of Onion Nuclei in Response to *Botrytis allii*. F. B. Kulfinski, Illinois State University, Normal, and A. J. Pappelis, Southern Illinois University, Carbondale.

Presumptive Vacuoles in Epidermal Nuclei of *Allium cepa*. F. B. Kulfinski, Illinois State University, Normal, and A. J. Pappelis, Southern Illinois University, Carbondale.

Cellular Senescence of Onion Leaf Base Outer Epidermal Cells. I. Changes in Nuclear Dry Mass and Kinetin Effect. Yi-shan Lin and A. J. Pappelis, Southern Illinois University, Carbondale.

Nuclear Degeneration in the Normal Epidermal Cell of Barley Leaf. I. Observation of Degenerating Process of Feulgen Technique. Hitoshi Kunoh and A. J. Pappelis, Southern Illinois University, Carbondale.

Section B

Room 104, Scovill Science Hall
Robert H. Mohlenbrock, Presiding
Southern Illinois University, Carbondale

Serologic and Disc Electrophoretic Investigation of Spore Proteins from selected Fern Taxa. Raymond Leo Petersen, University of Illinois, Chicago Circle.

Miocene Woods from Colorado. Elizabeth Wheeler, Southern Illinois University, Carbondale.

On the Structure of *Cordaites felicis* Benson from the Lower Pennsylvanian of North America. Charles W. Good, University of Illinois, Chicago Circle.

Bisporangiate Arborescent Lycopsid Fructifications from the Lower Pennsylvanian of North America. Sheila D. Brack, University of Illinois, Chicago Circle.

Additional Observations of *Conostoma anglo-germanicum* and *C. oblongum* from the Lower Pennsylvanian of North

America. Gar W. Rothwell, University of Illinois, Chicago Circle.

Applications of the Scanning Electron Microscope in Paleobotany. Michael A. Millay and Thomas N. Taylor, University of Illinois, Chicago Circle.

A Comparative Study of the Influence of Temperature on the Rate of Shoot Development of *Trientalis borealis*. Roger C. Anderson, Southern Illinois University, Carbondale.

The Climatic Tension Zones of Illinois and their Bearing on Plant Distribution. Donald Ugent and Roger C. Anderson, Southern Illinois University, Carbondale.

Vegetation of the Cretaceous Springs of Pope County, Illinois. John Schwegman, Southern Illinois University, Carbondale.

A Preliminary Report on Heron Pond, Johnson County, Illinois. John White, Southern Illinois University, Carbondale.

Analysis of the Grass Flora of Illinois. Robert H. Mohlenbrock, Southern Illinois University, Carbondale.

Chemistry

Section A

Room M-222, Liberal Arts Hall
William J. Probst, Chairman
Southern Illinois University, Edwardsvllie
Emanuel Merdinger, Presiding
Roosevelt University, Chicago

Composition of Hydrocarbons and Alcohols of Human and Animal Tissue Lipids. Leon L. Gershbein and Eric J. Singh, Northwest Institute for Medical Research, Chicago.

Metal Ion-Binding of some Amino Acids Related to Bacterial Spores. Lily Chung, E. Merdinger, K. S. Rajan, and N. Grecz, Roosevelt University and Illinois Institute of Technology, Chicago.

Isolation and Identification of Trehalase from *Pullularia pullulans*. Charles F. Lange and E. Merdinger, Hektoen Institute of Cook County Hospital and Roosevelt University, Chicago.

The Effect of Topical Anasthetics Proparacaine HCl, Benoxinate HCl, and Tetracaine HCl on growth of *Debaryomyces hanseni* and *Pullularia pullulans*. Emanuel Merdinger, Roosevelt University, Chicago. Studies on Manganese and Copper in Certain Neurological Diseases. Eleanor Berman, Hektoen Institute of Cook County Hospital, Chicago.

Effect of Amino Acid Analogs on Bacteriophage Multiplication in Parafluorophenylanine-Treated *Escherichia coli*. S. N. Millner and S. B. Binkley, University of Illinois, College of Medicine, Chicago.

Studies on the Properties and Specifications of Autoantibodies against Glomerular Basement Membrane in an Experimental Model. Ulrich H. Rudofsky and Raymond W. Steblay, University of Chicago, Chicago.

Fluorometric Analysis of Serum and Tissue Lipids on a Thin Layer Chromatogram Scanner. Donald Silverman, A. Dubin, and F. Steigmann, Hektoen Institute, Chicago.

Use of Solvent Perturbation in the Study of Pyruvate Kinsase. Cecil Davis and Jacklyn B. Melchior, Roosevelt University and Chicago College of Osteopathy, Chicago.

Blood Chemistry Profiles on Hospitalized Patients. M. I. Malik, G. E. Batayias, E. R. Tucker, R. A. Scheidt, and R. A. Collins, Department of Pathology, St. Luke's Hospital, Milwaukee.

Methods of Lead Intoxication Detection. K. Soo, R. A. Scheidt, and R. A. Collins, Department of Pathology, St. Lukes Hospital, Milwaukee.

Renal Calculi and Gall Stone Analysis using Infrared Spectrophotometry. R. Gregorski, R. A. Scheidt, and R. A. Collins, Department of Pathology, St. Luke's Hospital, Milwaukee.

Section B

Room M-224, Liberal Arts Hall
Ralph L. Bain, Presiding
Southern Illinois University, Edwardsville

The Spectrophotometric Determination of Cobalt Utilizing Sodium 2-Nitroso-1-Naphthol-5-Sulfonate as Applied to Stoddard County, Missouri Soils. William A. Gifford and Charles E. Williams, Southeast Missouri State College, Cape Girardeau, Missouri, and Albert L. Caskey, Southern Illinois University, Carbondale.

Spectra of Alkyl Acetoacetates and Their Copper Chelates. Carl Zirk, Morton, Illinois, and Forrest J. Frank, Illinois Wesleyan University, Bloomington.

Aryl Acetoacetates and their Copper Chelates: Spectra and Tautomerism.

Forrest J. Frank, Illinois Wesleyan University, Bloomington, and Terry K. Reid, Fontana Elementary School, Fontana, Wisconsin.

The Infrared Spectra of Some Copper and Silver Amino-Pyridine Complexes. S. Feldman and R. L. Bain, Southern Illinois University, Edwardsville.

What is a Straight Line? Boris Musulin, Southern Illinois University, Carbondale.

Inorganic Nitrogen Fixation: Studies on the Mechanism of the Reaction of Nitrogen with Titanium Cyclopentadienyl Complexes. Walter S. Guthmann and Mary L. Michielsen, Roosevelt University, Chicago.

Analysis of Monofluorophosphates Using the Fluoride Ion Electrode. David G. Rands and William Powell, Southern Illinois University, Edwardsville.

Highly Acidic Polyhalogenated Alcohols. Ralph M. Schure, Swift and Company, Oakbrook.

A Kinetic Study of Alkylation of 111° Amines with Ethyl Bromoacetate. Robert C. Duty and Ralph L. Gurnea, Illinois State University, Normal.

Synthethis of some 2, 5-Morpholinediones. Emil F. Jason and Jack Ballinger, Southern Illinois University, Edwardsville.

Chemistry Collegiate Section

Room M-226, Liberal Arts Hall
Carl Weatherbee, Chairman
Millikin University, Decatur

The Synthesis of Quaternary Ammonium Cyclic Anhydrides, Jack Roseman, Ken Hanck, Jerry Jordan, and Robert C. Duty, Illinois State University, Normal.

Polarographic Studies on the Dissociation Reaction of 2, 6-Dicarboxypiperidineacetocadmate (II) Complexes, David M. Coleman, T. J. Bydalek, R. E. Van Atta, Southern Illinois University, Carbondale. (This work was performed by Mr. Coleman at the University of Minnesota, Duluth, last summer under the direction of Dr. Bydalek through an arrangement by Dr. Van Atta.

An Inexpensive Student-Constructed Conductance Bridge. Richard J. Domalakes and R. E. Van Atta, Southern Illinois University, Carbondale.

A Kinetic Study of Tris (tetrammine-u-dihydroxo-cobalt (III) cobalt (III) Sulfate, Joseph Lauher and Wendell W. Hess, Illinois Wesleyan University, Bloomington.

A Study of α, ω — Bistri-n-butylphosphonium Bromides. Dan Rota, Forrest J. Frank, Illinois Wesleyan University, Bloomington.

Computer Calculations of X-ray Diffraction Patterns, Janie Snell, David Braught, Illinois Wesleyan University, Bloomington.

Kinetics of Hydrolysis of Monofluorophosphoric Acid in 50% Methanol. Dennis D. Staley, and David G. Rands, Southern Illinois University, Edwardsville.

Synthesis of Vinylene Carbonates, Lorraine Wright, Fred W. Breitbeil, III, DePaul University, Chicago.

Mannich Condensations of Thiophenols. Gregory C. Munie and Carl Weatherbee, Millikin University, Decatur. (Mr. Munie is a student at St. Procopium College but he did this research at Millikin University.

Conservation

Room 320, Scovill Science Hall
Harold E. Balbach, Chairman
Eastern Illinois University,
Charleston

Reintroduction of Native Game Animals in Illinois. Richard D. Andrews, Department of Zoology, Eastern Illinois University, Charleston.

Ecology of a Hillside Marsh in Coles County. Hampton Parker, Eastern Illinois University, Charleston.

The Illinois Nature Preserves System. W. D. Klimstra, Cooperative Wildlife Research Laboratory, Southern Illinois University, Carbondale. (Presented by George Fell).

The Private Estate as a Conservation and Research Area. Robert A. Bullington, Northern Illinois University, DeKalb.

Floristic Survey of the Rocky Branch Natural Area, Clark County. Gerald Hellinga, Eastern Illinois University, Charleston.

Can We Preserve the Reavis Spring Hill Prairie? John Warnock, Western Illinois University, Macomb, and Harold Balbach, Eastern Illinois University, Charleston.

Feasibility of Rearing and Restocking the Prairie Chicken in Illinois. Hurst H. Shoemaker, University of Illinois, Urbana.

Successional Patterns in Diverted Croplands of Crawford County. Norman Tracy, Newton High School, Newton.

Fire as a Hazard to the Prairie Fauna. Lewis J. Stannard, Illinois Natural History Survey, Urbana.

Dieldrin Levels in Eggs of Wild Pheasants in Illinois. Robert E. Greenberg, Illinois Natural History Survey, Urbana.

What is Goose Lake Prairie? Robert Betz, Northeast Illinois State College, Chicago.

Problems in Acquiring a Prairie in the Prairie State. Arnold Kugler, Illinois Department of Conservation, Springfield.

The Thomson-Fulton Scientific Study Area. Robert A. Evers, Illinois Natural History Survey, Urbana.

A Hill Prairie in Eastern Illinois Fifty Years Later. Constance Muccianti and Carol Pratt, Eastern Illinois University, Charleston.

A Record of the Past — Heron Pond. Roger C. Anderson, Southern Illinois University, Carbondale.

Life History Studies of Reptiles and Amphibians — Where? Edward Moll, Eastern Illinois University, Charleston.

The Position of Conservationists in the Allerton Park — Oakley Reservoir Controversy. Donald F. Hansen, Illinois Natural History Survey, Urbana.

Geography

Clyde Browning, Co-chairman
Millikin University, Decatur
and
John A. Jakle, Co-chairman
University of Illinois, Urbana

Section A

Room W-18, Liberal Arts Hall
John A. Jakle, Presiding

Areal Needs of Urban Areas in the Wabash Valley to 2020. Benjamin Moulton, Indiana State University, Terre Haute, Indiana.

Some Comments on the Spatio-Temporal Characteristics of a Population Potential Surface. Daniel J. Old and Eric G. Moore, Northwestern University, Evanston.

A Factorial Ecology of Rockford, 1960. Gerald F. Pyle, University of Chicago, Chicago.

Population Change Mechanisms Among the Old Order Amish Since World War II. James E. Landing, University of Illinois at Chicago Circle, Chicago.

Changes in the Distribution of School and School Enrollment in Massac County, Illinois, 1948-1968. Phillip D. Phillips, University of Illinois, Urbana.

Section B

Room W-19, Liberal Arts Hall
Nelson R. Nunnally, Presiding
University of Illinois, Urabana

Empirical Regularities in the Karstlands of Southeastern Illinois, Placido D. LaValle, University of Illinois, Urbana.

Two-Dimensional Syntax and Visual Cues in Aerial Photographs. John F. Betak, Northwestern University, Evanston.

Computer Assisted Instruction in Geography. Frank Jordan, Northwestern University, Evanston.

An Evaluation of Some Common Practices in Central Place Statistical Analysis. William Rabiega, Southern Illinois University, Carbondale.

Industrial Parks: A Quantitative Analysis of Selected Characteristics. Ronald J. Swager, University of Illinois, Urbana.

Consumer Travel, A Function of Price. Sam Liebovich, Bradley University, Peoria.

Soil-Plant Geography in Guyana. Clifford H. Simonson, Northern Illinois University, De Kalb.

Section C

Room E-300, Liberal Arts Hall
Martin W. Reinemann, Presiding
Northern Illinois University, DeKalb

A Simple Approach to the Analysis of the Locational Pattern of Electric Generating Plants in the United States. David G. Adams, University of Illinois, Urbana.

Animal Domestication as a Contemporary Phenomenon. Cornelius J. Loeser, Northern Illinois University, DeKalb.

The Native Fishing Industries of the Coast of Tanzania. Charles S. Alexander, University of Illinois, Urbana.

The Pre-canning Antiquity of Sweet Corn. Paul B. Frederic, Illinois State University, Normal.

Section D

Room W-18, Liberal Arts Hall

Gerald R. Showalter, Presiding

Ball State University, Muncie, Indiana

Some Recent Economic Developments in Tropical Australia. Allen R. McNeill, Illinois State University, Normal.

The Process of Industrial Development in an Underdeveloped Country: The Case of Columbia. Michael E. Sullivan, University of Illinois, Urbana.

Recent Developments in Mexico's National Border Program. C. Daniel Dillman, Northern Illinois University, DeKalb.

Jews of Costa Rica, Warren Fish, University of Illinois, Urbana.

Section E

Room W-19, Liberal Arts Hall

Robert B. Douglas, Presiding

Bradley University, Peoria

A Proposal to Use St. Clair County, Illinois Strip Mine Spoils for St. Louis Waste. James G. Bridwell, Southern Illinois University, Edwardsville.

New Iron Mining Operations in Missouri: Pea Ridge and Pilot Knob. Bruce A. Goetz, Southern Illinois University, Edwardsville.

Economic Geography of the Illinois Clay Products Industry. Robert L. Major, Illinois State Geological Survey, Urbana.

GEOLOGY

Room 115, Scovill Science Hall

I. Edgar Odom, Chairman

Northern Illinois University, DeKalb

Paleoecology and Paleontology of a Chaetetes Reef in Madison County, Iowa. David A. DeVries, Wheaton College, Wheaton.

Paleoecology of the Browns Town and Montpelier Formations (Oligocene-Miocene) of Jamaica. Robert J. Wallace, Northern Illinois University, DeKalb.

Caprock and Reservoir Potential of the Eau Claire in Northern Illinois. W. C. Lucas, Northern Illinois Gas Co., Aurora.

Stratigraphic Relations of the St. Laurent Limestone (Middle Devonian of Southeastern Missouri. Roger B. Nance, Illinois State Geological Survey, Urbana.

Geology of the West Range, Lincoln County, Nevada. Michael B. Lamport and John K. Winter*, University of Illinois, Urbana.

Regional Significance of Lithofacies of the Mesozoic Rocks of the Sand Springs Range, West Central Nevada. Konrad J. Banaszak, Northwestern University, Evanston.

Sedimentologic Characteristics of a Synorogenic Arenite, Churchill County, Nevada. Thomas A. Jones, Northwestern University, Evanston.

Scapolitization of Part of the Humbolt Igneous Complex, Nevada. James R. Riehle, Northwestern University, Evanston.

Interpretation of Compositional and Structural Data from the Red Hill Pluton, New Hampshire. William B. Size, University of Illinois, Urbana.

Petrology of the Northern Light Gneiss, Northern Light Lake, Thunder Bay District, Ontario. Michael G. Mudrey, Northern Illinois University, DeKalb.

Contact Metamorphism of Siliceous Limestone and Dolomite, Inyo Mountains, California. Charles H. Simonds, University of Illinois, Urbana.

An Estimate of Biasness in Geologic Outcrops. C. John Mann and Peter J. Poletti*, University of Illinois, Urbana.

Speculation on the Origin of the Paha Topography of Northwestern Illinois. Ronald G. Vail, Erie Community High School, Erie.

High Level Glacial Outwash in the Driftless Area of Northwestern Illinois. H. B. Willman and John C. Frye*, Illinois State Geological Survey, Urbana.

Explanations of Origin of Glacial Drift in Illinois, 1866-1890. Patricia M. Moran, Illinois State Geological Survey, Urbana.

Application of ^{14}C Analysis to Soil Genesis Studies. Thomas M. Ballagh* and E. C. A. Runge, University of Illinois, Urbana.

Carbonate Host Rock Alteration, Zn-Pb District of Northwestern Illinois, Duane M. Moore, Knox College, Galesburg.

Description of Pennsylvanian and Valmeyeran (Middle Mississippian) Strata in a Deep Diamond Drill Core at Springfield, Sangamon County, Illinois. William H. Smith and James W. Baxter, Illinois State Geological Survey, Urbana.

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* Speaker

Meteorology and Climatology

Room W-237, Liberal Arts Hall
Glenn E. Stout, Chairman
Illinois State Water Survey, Urbana

The Prediction of Forest Fire Hazard through Moisture Budgeting. Vernon Meentemeyer, Southern Illinois University, Carbondale.

The Prediction of Wilting Hazard in Plants through the Inspection of Optical Properties of Plant Leaves by Remote Sensing. Michael R. Boegler, Southern Illinois University, Carbondale.

The Effective Climate at the Alpine Timberline in Colorado. David M. Sharpe, Southern Illinois University, Carbondale.

Spatial Distribution Characteristics of Rainfall Rates in Illinois. William L. Shipp and F. A. Huff, Illinois State Water Survey, Urbana.

Fall Velocities of Small Water Droplets in Still Air. Robert Cataneo and Richard G. Semonin, Illinois State Water Survey, Urbana.

Relationships between the Radioactivity in Surface Air and in Precipitation. Wayne E. Bradley, Illinois State Water Survey, Urbana.

Synoptic Weather Producing Severe Thunderstorms in Central Illinois on 15 May 1968. John W. Wilson, Illinois State Water Survey, Urbana.

Rain and Hail on 15 May 1968. Stanley A. Changnon, Jr. and Glenn E. Stout, Illinois State Water Survey, Urbana.

Tornadoes on 15 May 1968. William L. Shipp, Illinois State Water Survey, Urbana.

Microbiology

Room 416, Scovill Science Hall
M. B. Linn, Chairman
University of Illinois, Urbana

White pine dwarf: Evidence for infectious casualty. H. H. Thornberry, J. J. Jokela and Mary Ruth Thompson, Departments of Plant Pathology and Forestry, University of Illinois, Urbana.

White pine infectious dwarf: Ultrastructure of needles. Mary Ruth Thompson, Department of Plant Pathology, University of Illinois, Urbana.

Isolation and characterization of a plant viral infection inhibitor from Stokes aster. A. L. Shawkat and H. H. Thornberry, Department of Plant Pathology, University of Illinois, Urbana.

The microorganisms transforming alumino-silicates. Peter Savostin, Department of Biological Sciences, Southern Illinois University, Edwardsville.

The crystalline formations of the silicates produced by soil fungi. Peter Savostin, Department of Biological Sciences, Southern Illinois University, Edwardsville.

Nylon mesh discs useful in the evaluation of soil fungitoxicants. O. N. Nesheim and M. B. Linn, Department of Plant Pathology, University of Illinois, Urbana.

Formation of pycnidia by *Diplodia zeae* in culture stimulated by nylon cloth on surface of media. J. L. McLaughlin and M. B. Linn, Department of Plant Pathology, University of Illinois, Urbana.

Physics

Room W-240, Liberal Arts Hall
Harry E. Tomaschke, Chairman
Greenville College, Greenville

The Ear as an Acoustic Instrument. Howard C. Roberts, University of Illinois, Urbana.

Pendulum Velocity Drive of Mossbauer Measurements. D. A. Cornell and S. A. Collins, Principia College, Elsah.

Quarks as Building Blocks. P. Scott Smith, Eastern Illinois University, Charleston.

The Crystal Structures of some Tetraphenylarsonium Compounds. R. J. Blattner and L. K. Walford, Southern Illinois University, Edwardsville.

An Inexpensive Photoelectric Effect Apparatus for the Introductory Physics Laboratory. R. Ellefson and S. Ward, Millikin University, Decatur.

Science Teaching

Room 212, Scovill Science Hall
Daniel G. Hance, Chairman
Cahokia High School, Cahokia

A Technique for Presenting a Theory of Aging. Harold A. Moore and John R. Carlock, Illinois State University, Normal.

A Technique for Demonstrating the Concept of Half-Life, Its Determination and Utilization. John R. Carlock and Harold A. Moore, Illinois State University, Normal.

Use of Balloon Models in Visualization and Prediction of Molecular Shapes. Dean L. Sinclair, Blackburn College, Carlinville.

Duplicating Industrial-Chemical Processes in the High School Laboratory in the Honor's Program. Gordon C. Bliss, Southern Illinois University, Edwardsville.

Room 214, Scovill Science Hall

Carob Seed and Culture. Louis J. Martens, Illinois State University, Normal. Novel Approaches in Teaching First Concepts in Science II: Arce the Arcella and Henry Harrison Owl. Edward M. Fashing, Chicago State College, Chicago.

A Modern Text in a Modern Chemistry Course. Shelba Jean Choate Musulin and Boris Musulin, Southern Illinois University, Carbondale.

A Decade of Science Seminars for Gifted High School Students. Soter George Kokalis, Chicago State College, Chicago.

Zoology

Section A

Room 401, Scovill Science Hall
Sidney Mittler, Chairman
Northern Illinois University, DeKalb

Fertility in Male White-Tailed Deer Fawns. Erich H. Follmann, Southern Illinois University, Carbondale.

Social Organization in a Captive Baboon Group *(Papio papio)*: A Preliminary Report. Gilbert K. Boese, Elmhurst College, Elmhurst.

Locomotor Responses of Adult House Mice to Olfactory Stimulation. Gerald Gaffney and W. D. Klimstra, Southern Illinois University, Carbondale.

Plasma Protein Deficiency Associated with a Ring C Chromesome, Microcephaly, and Severe Mental Retardation. William L. Daniel, Illinois State University, Normal.

Magnesium and Maganese ions as Protection Against X-ray Induced Dominant Lethal Mutations in *Drosophila melanogaster*. Richard J. King and Sidney Mittler, Northern Illinois University, DeKalb.

Ionic Protection of X-ray Induced Dominant Lethals in *Drosophila melanogaster*. Kenneth E. Balkin and Sidney Mittler, Northern Illinois University, DeKalb.

Serological Studies with the Domestic Chicken II Electrophoretic Separation of Plasma Proteins. Eugene Braun and Jack Bennett, Northern Illinois University, DeKalb. W. Elwood Briles, DeKalb AgResearch Inc., DeKalb.

Electrophoretic Analysis of Plasma Proteins of the Mallard. Richard C. Owen and Jack Bennett, Northern Illinois University, DeKalb.

Serological Studies with the Domestic Rabbit III. Proteins of Serum and intraocular fluids. Robert Andrini and Jack Bennett, Northern Illinois University, DeKalb.

Chromatographic Comparison of Pteridines in White Locus Alleles of *Drosophila melanogaster*. II Sequential Quantitative Changes Produced by the White-Apricot Allele. Katherine W. Bennett and Jack Bennett, Northern Illinois University, DeKalb.

Behavioral Genetics of *Drosophila melanogaster*. IV. Study of Traits Associated with the Single Gene Substitution *w* for *w+*. Jack Bennett and Martha Anne Walke, Northern Illinois University, DeKalb.

Immunological Function Study of Bursa of Fabricius in Chicken. Robert Elde, William Werner, Dana Johnson and E. J. Kennedy, North Park College, Chicago.

Section B

Room 402, Scovill Science Hall
Lester C. Shell, Presiding
Milikin University, Decatur

Cellular Responses of the Laboratory Mouse to Experimental Infection with Larvae of *Gnathostoma procyonis* (Nematoda: Spirurida). Harry W. Huizinga and Mary Ann Oblak, Illinois State University, Normal.

Description of *Splendidofilaria passerensis* n. sp., a filarial worm from the English sparrow, *Passer domesticus*. Carl F. Koch, Illinois State University, Normal.

In Vitro Cultivation of several Schizogonous Stages of *Eimeria stiedae* (Lindemann, 1865). Lawrence J. Blecka, Southern Illinois University, Carbondale.

The Development of Temperature Regulation in the Prairie Vole *Microtus ochrogaster*. Philip Kastner, Triton College, Northlake and V. C. Pederson, Western Illinois University, Macomb.

Electroanesthesia and Anoxia. R. S. Pozos, A. W. Richardson, L. E. Strack and H. M. Kaplan, Southern Illinois University, Carbondale.

Electrosleep, Electroshock and Lithium Chloride, Possible Common Mode of Action. R. S. Pozos, L. E. Strack, A. W. Richardson and H. M. Kaplan, Southern Illinois University, Carbondale.

Studies Concerning the Existence of Functional Accessory Parathyroid Tissue in *Rattus norvegicus*. James M. Goldinger, Lake Forest College, Lake Forest and Vernon C. Pederson, Western Illinois University, Macomb.

Effect of Ingested Gossypol on the Duodenum of Rats. Jim N. Tone and D. Reed Jensen, Illinois State University, Normal.

Histological Studies of the Nephridium and Pericardial Lining of *Quadrula nodulata*. Paul Robert Myers, Illinois Wesleyan University, Bloomington.

Observations on the Ecology of Desert Snails of the Genus *Micrarionta*. Hugh Rawls, Eastern Illinois University, Charleston.

THE ANNUAL BUSINESS MEETING

President Ashby called the Business Meeting to order at 12:35 p.m. at the Redwood Restaurant. Approximately 120 Academy members were present.

Reports of Officers. — Dr. Musulin distributed copies of the Treasurer's Annual Report. Dr. Wantland moved, seconded by Dr. Bennett, to accept the Treasurer's Annual Report.

Milton D. Thompson presented the Librarian's Report.

Reports of Standing Committees. — Dr. Rands reported the work of the Research Grants Committee and moved that the following grants be awarded:

Gar W. Rothwell—$100.00 University of Illinois, Chicago Circle. Travel and shipping costs.
Sheila D. Brack—$100.00 University of Illinois, Chicago Circle. Travel and shipping costs.
Gordon R. Thurow—$100.00 Western Illinois University. Travel and commodities.
Boris Musulin—$100.00 Southern Illinois University. Student wages.
Benedict J. Jaskoski—$200.00 Loyola University. Supplies and wages for assistant.
Gus A. Pappelis—$175.00 Carbondale Community High School, G. L. Cuendet, Principal. Equipment and commodities.
Robert D. Sholl—$160.00 Western Illinois University. Equipment.

The motion was seconded by Dr. Merdinger and passed.

President Ashby reported on the Budget which had been approved by the Council. Dr. Fisher moved, seconded by Dr. Liberta, to accept the report of the Budget Committee. Motion passed.

Dr. Bennett, Chairman of the Membership Committee, moved to install into the Academy, with all rights and privileges, all members who joined since the last business meeting. The motion was seconded by Dr. Kanatzar and passed. Academy membership stands at 1,132.

Reports of Special Committees. — Dr. G. R. Yohe presented a report on the progress of the Permanent Central Office Committee. The proposal has been approved by vote of the Council and awaits the outcome of the balloting of the Illinois Junior Academy of Science Officers to determine whether or not support from foundations can be sought with the proposal in its present form.

Milton D. Thompson, Chairman of the Nominations Committee, presented the report of the Nominations Committee. President Ashby called for nominations from the floor. There were no further nominations. Dr. Fisher moved, seconded by Dr. Bennett, that the report of the Nominations Committee be accepted and the Secretary be directed to cast a unanimous ballot of approval. Motion approved. A list of the officers, standing committee chairmen, and section chairmen for 1969 - 1970 is included herewith.

Joan Hunter, Chairman of the Planning Committee, introduced the proposed constitutional amendments for a vote by the members present. All of the proposed amendments were passed. These amendments are listed below:

Article II OBJECTS

2. No substantial part of the activities of the Illinois State Academy of Science shall be devoted to any but scientific and/or educational objectives.
3. The net assets of the Illinois State Academy of Science shall be used for and only for, scientific and/or educational purposes and shall not in any way accrue to any group or private individual. The funded property shall be administered and operated in such manner that it will at all times maintain a tax exempt status under the provision of the United States Inter-

nal Revenue Code, both for income tax and gift tax purposes.

4. In the event of dissolution of this corporation all of its assets shall be distributed by the Council in accordance with Section 45 and/or Section 55 (c) of the General not for Profit Corporation Act - 1963, to one or more organizations exempt under Section 50 1 (c) (3) of the Internal Revenue Code as here after amended or as it may be supplanted by another section under which this corporation is granted exemption under the Internal Revenue Code for income tax purposes.

Dr. Harold M. Kaplan, Chairman of the Resolutions Committee, submitted the following resolutions; all were approved by the members of the Academy:

1968-69

RESOLUTION NUMBER ONE:
APPRECIATION TO HOST

Whereas many members of the Administration, Faculty and Staff of Millikin University have been responsible for the arrangements essential to the Sixty-second Annual Meeting of the Illinois State Academy of Science.

Be it resolved that the Academy express its gratitude to all who have participated in any way in arranging this Annual Meeting, and especially to: Dr. Paul L. McKay, President of the University, Dr. Carl Weatherbee, Second Vice President of the Academy, in charge of local arrangements, Mr. Gerald Redford, assistant to the President, and William Carey, Director of Public Relations of Millikin University.

Be it further resolved that the Secretary of the Academy be directed to send copies of this resolution to those specifically named.

RESOLUTION NUMBER TWO:
APPRECIATION OF SERVICE

Whereas responsibility has been accepted by Dr. Robert A. Evers, Illinois Natural History Survey, Urbana, Dr. Randy Boggess, University of Illinois, Urbana, and Dr. Charles Kendeigh, University of Illinois, Urbana, for arranging the Botany Field Trip, and

Whereas these Field Trips constitute a significant portion of the program of the Sixty-second Annual Meeting of the Academy,

Be it resolved that the Academy express its thanks to these individuals for organizing and conducting the Field Trips, and

Be it further resolved that the Secretary of the Academy be directed to send a copy of this resolution to those specifically named.

RESOLUTION NUMBER THREE:
PRESERVATION OF
GOOSE LAKE PRAIRIE

Whereas many remaining natural areas of this State are threatened with destruction, and

Whereas the Illinois State Academy of Science is deeply concerned with the preservation of natural areas, and

Whereas the State of Illinois is currently interested in purchasing and preserving the Goose Lake Prairie, a unique site that should be maintained for scientific study and for the enjoyment of future generations,

Be it resolved that the Academy support the efforts now underway for the preservation of this area, and

Be it further resolved that the Secretary of the Academy send copies of this resolution to Governor Richard B. Ogilvie, and to Mr. William Rutherford, Director of the Department of Conservation of the State of Illinois.

RESOLUTION NUMBER FOUR:
PRESERVATION OF
ROBERT ALLERTON PARK

Whereas Robert Allerton Park, administered by the University of Illinois, is a natural area of outstanding scientific, educational and esthetic importance, and

Whereas the U. S. Army Corps of Engineers proposes the construction of the Oakley Dam and Reservoir which would permanently flood the lowland forested areas of this Park, which are of considerable scientific interest, and

Whereas certain alternate plans have been put forth which would eliminate the permanent flooding of the Robert Allerton Park and

Whereas a proposal to construct the dam such that the pool level does not exceed 621 feet would not seriously affect the value of the Reservoir or significantly affect the Park, therefore

Be it resolved that the Academy recommend preservation of Allerton Park through establishment of a maximum level for the pool or by such other plans that would maximize the conservation of the Park and its resources, and

Be it further resolved that the Secretary of the Academy send copies of this Resolution to the following persons:

Governor Richard B. Ogilvie
Dr. David Dodds Henry, President of the University of Illinois
Mr. William Rutherford, Director, Department of Conservation of the State of Illinois
Senator Everett M. Dirksen, Washington, D. C.
Senator Charles H. Percy, Washington, D. C.
Representative William Springer, Washington, D. C.

RESOLUTION NUMBER FIVE: NECROLOGY

Whereas during the past year the Academy has lost by death:

Clemens Chambers, Chicago
Lyle W. Finley, Monmouth
Mabel Clark Paterson, Charleston
Clarence P. Schumers, Lemont
Walter M. Scruggs, Charleston

Be it resolved that the Academy give expression to its sorrow for the deceased members by rising for a moment of silence.

Kenneth H. Harmet
Secretary

OFFICERS AND STANDING COMMITTEE NOMINEES FOR 1969-1970

President: Harold M. Kaplan, Southern Illinois University, Carbondale.
First Vice-President: Anthony E. Liberta, Illinois State University, Normal.
Second Vice-President: Charles K. Hunt, University of Illinois, Chicago Circle, Chicago.
Secretary: Kenneth H. Harmet, Northern Illinois University, DeKalb.
Treasurer: Boris Musulin, Southern Illinois University, Carbondale.
Librarian: Paul W. Parmalee, Illinois State Museum, Springfield.
Editor: Malcolm Jollie, Northern Illinois University, DeKalb.
President, Junior Academy: Sister Mary Alvernia, O.S.F. Madonna High School, Chicago.

THE COUNCIL

Immediate Past President: William C. Ashby, Southern Illinois University, Carbondale.

Elected Councilors:

E. C. Galbreath (to 1970), Southern Illinois University, Carbondale.
Donald F. Hoffmeister (to 1970), Museum of Natural History, University of Illinois, Urbana.
G. R. Yohe (to 1971), State Geological Survey, Urbana.
William J. Probst (to 1971), Southern Illinois University, Edwardsville.
David G. Rands (to 1971), Southern Illinois University, Edwardsville.
Charles K. Hunt (to 1971), University of Illinois, Chicago Circle, Chicago.
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TREASURER'S ANNUAL REPORT —
JAN. 1 - DEC. 31, 1968

Receipts & Disbursements

Cash Receipts			
Dues (Membership 1968 - 1132)		$	5,147
Regular	$ 4,563		
Student	206		
Emeritus	23		
Contributing	155		
Life	200		
Library Subscriptions			431
A.A.A.S. Research Grants			506
Annual Meeting Fees			439
Interest Income			1,057
U. S. Government Ob.	287		
Savings & Loan Ac.	770		
Sale of Publications		$	2
Total Receipts			$ 7,582
Cash Expenditures			
Research Grants			1,337
Council			804
Editor's Office			171
Frank H. Reed Mem. Prize			75
Honoraria			500
Librarian			617
Membership Committee			421
Science Talent Search			478
Secretary's Office			777
Treasurer's Office			658
Miscellaneous			118
Total Expenditures			5,956
Excess of Receipts over disbursements			1,626

TOTAL ASSETS

Reserve Fund			
Cash in Bank		6,446	
Investments		19,500	
U.S. Gov. Ob.	4,500		
Savings & Loan Ac.	15,000		
Total Reserve Fund			25,946
Permanent Fund			
Investments		2,550	
U.S. Gov. Ob.	2,000		
Savings & Loan Ac.	550		
Total Permanent Fund			2,550
Frank H. Reed Memorial Fund		1,472	1,472
Total Assets			$ 29,968
Total Liabilities & Equities			$ 29,968

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For publication in the *Transactions*, articles must present significant material that has not been published elsewhere. Review articles are excepted from this provision, as are brief quotations necessary to consider new material or varying concepts. All manuscripts must be typewritten, double spaced, with at least one-inch margins. The original copy and one carbon copy should be submitted.

Titles should be brief and informative. The address or institutional connection of the author appears just below the author's name. An abstract must accompany each article. Subtitles or center headings should be used; ordinarily one uses subtitles such as *Materials*, *Methods*, *Results*, *Discussion*, *Summary*, *Acknowledgments*, and *Literature Cited*.

No footnotes are to be used except in tables.

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Malcolm T. Jollie,
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